SCIENTIFIC POSTER SESSION SCHEDULE

Posters of the accepted abstracts can be viewed in Hall A of McCormick Place, on Tuesday, July 31 and Wednesday, August 1. All posters will be posted from 9:30am until 5:00pm. Presenting authors will be in attendance from 12:30pm until 1:30pm. Please refer to the onsite Abstracts Title Guide for a complete schedule of posters.

Below are the topics and their scheduled times.

TUESDAY, JULY 31, POSTER SESSIONS

9:30am – 5:00pm

WEDNESDAY, AUGUST 1, POSTER SESSIONS

9:30am – 5:00pm

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Tuesday, July 31, 2018

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

Cancer/Tumor Markers

A-001

Lot-to-Lot Consistency of Abbott Tumor Marker Assays

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Background: Serological tumor marker assays are a valuable tool, aiding clinicians in the prognosis and management of cancer patients. For patients being monitored with tumor marker assays, changes in values can have significant implications for therapy or intervention. It is therefore imperative that the analytical performance of these assays remain consistent from one lot of reagent to the next to impart confidence that fluctuations in values are due to changes in the patients' tumor status and not to reagent lot-to-lot variability. Objective: The goal of this study was to evaluate the lot-to-lot performance of eight tumor marker assays (CA19-9, CA15-3, CEA, AFP, Total PSA, Free PSA, CA125 and HE4) over an extended period of time. Methods: Quality controls (QC) with values across the measurement range and human serum panels targeted at or near medical decision points were tested on each new reagent lot manufactured over a nine- to 33-month period. Assays were run on the Abbott Architect. For CA19-9, CA15-3, CA125 and HE4, QC was tested in replicates of two (HE4), five (CA15-3 and CA125), or 10 (CA19-9) and panels were tested in singlicate in one run on one instrument at time of manufacture. For CEA, Total PSA, and Free PSA, QC and panels were tested in replicates of 15 in two runs on one instrument. AFP QC was tested in replicates of 15 in two runs, with each run on a different instrument. For all assays, multiple calibrator lots, instruments and, in some cases, panel lots were used over the duration of analysis. Imprecision (percent coefficient of variation (%CV)) was calculated across all reagent lots on each control and panel level mean for each assay. Results: The imprecision for each control value for all assays was less than or equal to 4.7%CV. Human serum panels at or near important medical decision points had the following imprecision (CV): HE4, ≤6.8%; CA15-3, ≤4.8%; CA125 ≤4.1%, CA19-9, ≤5.2%, Total PSA, ≤4.7%; Free PSA, ≤4.0%; and CEA ≤2.8%. Conclusions: Each of the eight tumor marker assays evaluated showed consistent lot-to-lot performance on all controls and human serum panels near important medical decision points. Reliable laboratory results give physicians confidence that changes in tumor marker values are reflective of a change in patient status and will lead to more informed treatment decisions.

A-002

Serum Free Light Chain Stability at -20°C, +4°C and +22°C

M. D. Coley, C. T. Wilson, K. L. Sharp, S. J. Harding. *The Binding Site, Birmingham, United Kingdom*

Introduction

Serum free light chain (FLC) assays aid in the diagnosis and monitoring of plasma cell dyscrasias and associated conditions. Freelite® assay reagents, calibrators and controls are subjected to extensive testing during product development to ensure that they are robust to changes in temperature encountered during shipping and storage. However, this stability cannot be assumed of FLCs in patient serum samples. In this study the stability of κ and λ FLCs in serum at -20°C, +4°C and +22°C was assessed using Binding Site Optilite® Freelite assays.

Method

Ten healthy adult donors were selected at random from a pool of consenting donors. Whole blood was obtained from these donors by venepuncture into BD Vacutainer™ SST™ serum separator tubes and centrifuged according to the manufacturer's instructions. The day of venepuncture was designated as 'day 0'. Vials containing 0.35µL of serum from each donor were separated into three groups and stored at either -20°C, +4°C or +22°C until testing; one vial from each donor was set aside for day 0 testing. κ and λ FLCs were measured on the Optilite analyser (The Binding Site Group Ltd., Birmingham, UK) using Freelite kappa and lambda assays (The Binding Site Group Ltd., Birmingham, UK). Assays were calibrated and validated as instructed in the product inserts. Each sample at each storage temperature was tested in triplicate at days 1, 2, 3, 4, 7, 10, 14, 22 and 28. On each day the results were re-validated with an unopened vial of reagent and compared to day 0 results with the Kruskall-Wallace statistical test with Bonferroni correction using the Analyse-it™ software package. A statistically significant difference (p-value <0.05) indicated sample instability.

Results

When results were compared to day 0, serum κ FLCs stored at -20°C and +4°C produced a p-value of >0.05 at each time-point. On day 7, at +22°C, there was a -24.8% change in the median sample result compared to day 0 (p-value <0.05), and a p-value of <0.05 was produced at each time-point thereafter. Serum λ FLCs produced a p-value of 1.0 for each temperature and time-point tested.

Conclusions

We conclude that κ FLCs are stable in serum for up to 28 days at -20°C and +4°C. However, κ FLC stability was only observed for up to 4 days at 22°C. λ FLCs are stable for up to 28 days at -20°C, +4°C or +22°C. These findings validate the directions given in the product insert, which states that "Samples may be stored at 2-8°C for up to 21 days, but for prolonged storage they should be kept frozen at -20°C or below". Knowledge of the stability of free light chains in serum is important so that laboratories are aware of the appropriate patient sample transport logistics to ensure sample integrity is maintained.

A-003

Evaluation of Des-gamma-Carboxiprothrombin (PIVKA II) as complement of serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in Mexican people

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Background: The 80% of hepatocellular cancer (HCC) is detected in the advanced stage in Mexico. Serum alpha-fetoprotein is preferred biomarker in HCC, nevertheless has a low sensitivity, specificity and positive predictive value. Some studies suggest the Des-gamma-Carboxiprothrombin (DCP) is a potential marker of HCC. Study aims to evaluate the predictive value of DCP test as complement of serum alpha-fetoprotein (AFP) for diagnosis of hepatocellular carcinoma in Mexican people. **Methods:** We conducted a retrospective study from November 2017 to January 2018. 56 patients with AFP> 10 ng/mL were included to measured DCP (PIVKA II Abbott ARCHITECT STAT assay). Demographic and clinical information were collected from the clinical file. The results of hepatic elastography and anatomic pathology were used to determine liver pathology (cirrhosis and HCC) or non-hepatic pathology. Sensitivity, specificity and positive predictive value (PPV) were calculated based on these results. For the reference intervals we used EP Evaluator and the cutoff point was established in 40 mAU/mL. **Results:** 14 patients had DCP <40 mAU/mL, none of them had HCC, one patient has cirrhosis and 13 other non-hepatic tumors. 62 patients had DCP >40 mAU/mL, of which 20 had HCC, 8 patients have cirrhosis and 14 other non-hepatic tumors. The sensitivity was 96.9%, the specificity 48.1% and the PPV 66.6%, which is indicative that if a patient has positive AFP and positive DCP has a 66.6% probability of having HCC. **Conclusion:** Our study support DCP determination as complement of serum alpha-fetoprotein as an important marker of probability of hepatocellular carcinoma in Mexican people and points the way for further cohort prospective clinical studies to explore it´s value in monitoring response to therapy and patient´s follow-up.<!--EndFragment-->

A-004

Hereditary Cancer Multi-gene Panel screening among Brazilian patients

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Background: Genetic panels have become powerful and accessible tests for screening germline variants, providing a source for diagnosis and medical guidance. Although there are several types of cancers already linked to hereditary predisposition with well-known genes as key players, such as *BRCA* genes and breast cancer, other secondary genes are also important for disease development and are not usually analyzed at first screening. Different hereditary cancer panels are currently commercially available, recommended for patients with familiar history of cancer. **Objectives:** Screen the prevalence of variants in medical reports in a hereditary cancer-related multi-gene panel among Brazilian patients through next generation sequencing (NGS). **Methodology:** We used an Ampliseq custom 40 genes panel to investigate variants in 49 pa-

tients with familiar history of cancer along the years of 2016 and 2017. Genomic DNA libraries were prepared from blood samples, following the manufacturer's instructions. Sequencing was performed on the Ion Torrent PGM™ or S5™ platforms. The sequence data were processed using standard Ion Torrent Suite™ Software. Reads were aligned to the human genome reference (hg19/GRCh37) with TMAP and variant calling were performed by Ion Reporter™ Software. Variants in the entire coding regions and 10 pb of exon boundaries with MAF <0.02 were filtered. Variant classification was performed according to ACMG-AMP guideline. Pathogenic, probably pathogenic and variants with unknown significance (VUS) were reported. **Results and Discussion:** There was a 4.4X increase in the number of tests from 2016 to 2017. Although cancer diagnoses are more common in men than in women, males and females represented respectively 22% and 78% of the patients. This may be due to more usual cancer screening in women, who seeks more contact with health professionals throughout their lives. The average age of males was higher (52.3 ± 13.5 years) than females (46.2 ± 11.5 years). Since men are usually less willing to talk about their health concerns, this may lead to a late screening and diagnosis. 33 patients (67%) presented at least one reported variant. A total of 62 distinct variants (56 SNPs and 6 INDELs) were found in 29 genes (*APC, ATM, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FANCC, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTCH1, PTEN, RAD51C, RECQL, RET, SMAD4* and *TP53*). 53 variants were classified as VUS and nine as pathogenic or probably pathogenic. From the VUS, 15 are novel variants found in 11 patients. *APC* was the most mutated gene with seven VUS in six patients. **Conclusions:** This study shows the importance of disease screening with multi-gene panels for patients with familiar history of cancer, as relevant variants could be frequently detected. Surveys of the reported variants may contribute to define their importance as prevention and diagnostic tools and guide future genetic counseling campaigns aiming early cancer detection. The annually increasing amount of samples will also significantly contribute to our knowledge about previous and new potentially pathogenic variants among the Brazilian population.

A-005

A 83-01 inhibits the epithelial-mesenchymal transition and increased invasion induced by platelets in ovarian cancer cells

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Background: Ovarian cancer is the leading cause of death among all gynecological malignancies. The high mortality is partly resulted from metastasis, mainly referred to peritoneal dissemination and hematogenous metastasis for ovarian cancer. It has been demonstrated that platelets play an important role in promoting metastasis in ovarian cancer. However, antiplatelet agents could not inhibit platelet-induced epithelial-mesenchymal transition (EMT) gene expression alterations. Therefore, there is an urgent need for more reliable drugs, which can abolish the effects of platelets on ovarian cancer cells. Many studies have shown that platelets enhance the metastatic activity of tumor cells through TGF-β/Smad pathway. However, whether A 83-01 (a TGF-β inhibitor) could inhibit the platelet-induced EMT and invasion in ovarian cancer cells remains unknown. **Objective:** In the current study, we sought to examine the possible role of A 83-01 in inhibiting platelet-related prometastasis in ovarian cancer cells. **Methods:** SK-OV-3 and OVCAR-3 cells were seeded in 6-well cell culture plates with appropriate culture medium. After adherence, cells in each well were treated with platelets, platelets + A 83-01 or A 83-01 alone, respectively. Cells without any treatments were used as controls. The EMT alterations in ovarian cancer cells at molecular level were assessed by EMT-related markers using quantitative real-time PCR (qPCR) and Western blot. Transwell assays were used to analyze the invasive capacity of the cell lines. **Results:** Analysis of mesenchymal markers and transcription factors involved in EMT revealed that the mRNA expression of snail, vimentin, N-cadherin, fibronectin (Fn1) and matrix metalloproteinase-2 (MMP2) was significantly up-regulated in both cell lines. Additionally, there was a significant down-regulated in the mRNA expression of epithelial marker E-cadherin in SK-OV-3. Western blot analysis revealed the reduced E-cadherin protein levels and increased N-cadherin protein levels in platelet-treated SK-OV-3 and OVCAR-3 cells. The protein levels of MMP2 were also increased in comparison with controls. Co-culture with platelets markedly increased the invasive properties of SK-OV-3 and OVCAR-3 cells. Specifically, platelets induced a 3.2-fold ($p < 0.0001$) and 3.1-fold ($p = 0.0004$) increase in SK-OV-3 and OVCAR-3 cells invasion, respectively. Upon addition A 83-01 to media from platelets-treated ovarian cancer cells, the EMT-like alterations were inhibited at the transcriptome level. A 83-01 treatment also restored the expression of E-cadherin and repressed that of N-cadherin and MMP2 in both cell lines at protein level. Concordantly, exposure of platelets-treated SK-OV-3 and OVCAR-3 cells to A 83-01 resulted in a 2.6- and 2.3-fold significant reduction in their invasion activity compared to those incubated with platelets alone, respectively. **Conclusion:** A 83-01 inhibits the EMT and increased invasion induced by platelets in ovarian cancer cells. The findings of the present study suggest that A 83-01 may be useful for establishment of therapies tailored to inhibiting ovarian cancer metastasis.

A-006

Insights into Analytical Validation and Clinical Utilization of Alternating Current Electrokinetics (ACE)-Based Assays for the Quantification of Cell-Free DNA

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The isolation and quantification of analytes in complex biological fluids continue to be challenging for researchers and clinicians studying the role of macromolecules in diseases such as cancer. We have applied a novel technology based upon Alternating Current Electrokinetics (ACE) to enable direct isolation and on-chip analysis of nanoparticles and macromolecules such as high molecular weight cell-free DNA (hmw cfDNA). The utilization of a simple and rapid workflow which does not require extensive purification and processing steps constitutes one of the primary advantages of this application of ACE technology. For our ACE-based assay that quantifies hmw cfDNA, blood is first processed to plasma via centrifugation. A fluorescent detection reagent is added to the plasma sample, which is then pipetted into a single-use cartridge containing a microelectrode array. The cartridge is inserted into a dedicated instrument which provides the appropriate mechanical, microfluidic, electrical, thermal, and optical control to enable capture and visualization of hmw cfDNA, and the resultant images are quantified via purpose-built software. We demonstrate the feasibility of assays based upon ACE technology to quantify DNA in both defined buffer systems and complex biological fluids such as plasma, with concentrations being determined via reference to calibrators with known concentrations of DNA. Assay parameters are still being optimized for specific assay formats, but prototype assays show a Limit of Detection (LoD) of <10 ng/mL and excellent linearity over a reportable range of at least two orders of magnitude. These analytical parameters enable the quantification of hmw cfDNA in the vast majority of clinical samples without dilution, and in one analysis of 177 plasma samples derived from cancer patients, at least 91% (162 samples) had hmw cfDNA concentrations within the reportable range. It has been known for some time that hmw cfDNA is present at elevated levels in the blood of cancer patients. Since many of these studies employed quantitative PCR (qPCR) as the methodology to determine cfDNA concentrations, we have conducted experiments to compare quantitative measurements of cfDNA by ACE-based and qPCR assays in a set of plasma samples processed from the blood of cancer patients and healthy normal donors. For one representative set of assays from this series of experiments, regression analysis comparing ACE-based and qPCR assays on 19 plasma samples from Non-Small Cell Lung Cancer (NSCLC) patients receiving systemic treatments resulted in an excellent degree of fit between the two assays ($R^2>0.9$). Initial results with another set of samples demonstrate that changes in hmw cfDNA levels over time are correlated to disease progression in a cohort of NSCLC patients. We are pursuing the use of ACE-based assays for monitoring cancer progression and also for early-stage cancer detection, and further development of the technology and assays are enabling its application to the isolation and quantification of other disease-relevant analytes.

A-007

Towards development of an exosomal protein biomarker signature to monitor cancer progression in uveal melanoma

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Uveal melanoma (UM) is a particularly rare type of non-cutaneous melanoma that arises from melanocytes in the uveal tract, forming a malignant tumor of the eye. Metastatic disease frequently involves the liver and is associated with a high rate of mortality, typically within 15 months. There is a significant deficit in our ability to diagnose early and effectively treat UM, which accounts for the poor outcomes. By advancing diagnostics to detect UM in a pre-metastatic state, we may be able to improve outcomes. Exosomes are membrane bound vesicles formed in multivesicular endosomes that carry proteins, genetic material, among other molecules, out of the cell through cellular secretion. Melanoma cell-derived exosomes appear to be intricately involved in initiation, growth, pre-metastatic niche formation, and subsequent metastasis of melanoma. Thus, we hypothesize that these molecules can be used as biomarkers to diagnose disease earlier. In addition, these vesicles are readily found in clinical samples and provide

a specific protein signature allowing for the development of a diagnostic assay. UM in particular is poised to benefit most from improved detection because of the difficulty of pre-metastatic detection and severely negative outcomes associated with late diagnosis. Despite the growing relevance of circulating exosomes as cancer biomarkers within the clinical community, their use has been limited largely due to a lack of efficient, straightforward isolation procedures that can be used in a clinical laboratory setting. We report here the development of a simple, quick, and inexpensive procedure for isolating urine exosomes. Our method requires no specialized equipment and we have established a standard operating procedure that can readily be adapted to clinical laboratories. Briefly, the first spin removes dead cells and cellular debris from the sample. A second higher speed spin removes larger extracellular vesicles, along with Tamm-Horsfall protein filaments. This pellet is subsequently chemically treated to remove the Tamm-Horsfall protein, thereby freeing the entrapped exosomes, and spun a third time to concentrate the exosomes. Successful isolation was confirmed by western blot analysis using six previously identified protein exosome markers: CD9, CD81, ALIX, HSP90b1, HSP1a1, and ACTN4. Comparison between native and processed urine revealed a robust enrichment of exosomes in the processed sample. We have also used an adaptation of this procedure with cell culture samples and have detected key signaling molecules, including mature integrin b1 and tyrosine kinase receptors, within UM exosomes. The presence of these molecules in exosomes yields insight into signal transduction pathways activated in the cancerous cells from which the vesicles are derived, and can be used to inform clinical decision making. This biomarker signature, comprised of exosomal proteins and signaling molecules, is a harbinger of aberrant processes in cancer progression. Detection of this biomarker signature in noninvasively obtained clinical samples, such as urine, can be used to monitor disease progression in real-time, thereby leading to more effective care for UM patients.

A-008

Novel Prognostic Scores Based on Plasma Prothrombin & Fibrinogen Levels in Patients with AFP-negative Hepatocellular Carcinoma

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Background: Non-invasive tools for the prognosis of and alpha-fetoprotein(AFP) negative hepatocellular carcinoma(HCC)are urgently needed. The present study proposed a prognostic system based on preoperative plasma prothrombin time(PT) and fibrinogen(Fbg) (PT/Fbg system). **Methods:** With respect to AFP-negative HCC, we compared the prognostic value in PT/Fbg system, Glasgow prognostic score (GPS) and aminotransferase (ALT)/aspartate aminotransferase (AST) ratio(LSR). The present study retrospectively analysed patient characteristics, clinicopathological factors, and the level of pretreatment biomarkers (PT, Fbg, albumin, C-reactive protein, ALT and AST) in 628 patients with CRC. **Results**: Patients with increased PT and Fbg levels were allocated a score of 2, patients with only one of these abnormalities were assigned score 1, and patients with neither of these abnormalities were allocated a score of 0. The discriminatory ability of the PT/Fbg system, GPS and LSR were assessed using an ROC curve.The optimal cut-off values of biomarkers were determined using ROC analysis, and albumin and C-reactive protein were estimated as described previously. The following distributions of the PT/Fbg system scores were observed: 187(29.78%) patients had a score of 0, 305(30.65%) had a score of1, and 134(22.69%) patients had a preoperative score of 2. The prognostic significance of the PT/Fbg system was determined using univariate and multivariate cox hazard analyses in AFP-negative HCC. Multivariate analysis revealed that patients with a higher PT/Fbg systemexhibited worse OS than patients with a lower PT/Fbg system. Furthermore, the PT/Fbg system was associated with treatment and exhibited a higher AUC value(0.684). **Conclusions**: These results suggest that the overall survival (OS) was shorter in AFP-negative HCC patients with a high PT/Fbg system. Therefore, our study proposes preoperative evaluation of the plasma PT/Fbg system to predict the OS of AFP-negative HCC patients.

A-009

Point of Care Dipstick Assessment of Ascitic Fluid: Comparism With Serum Ascites Albumin Gradient (SAAG) in Differentiation of Ascitic Fluid Into Exudate and Transudate.

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Background: Excessive ascitic fluid accumulation in the peritoneal cavity is seen as a clinical problem in all parts of the world by many doctors. This study attempts to differentiate exudate and transudate ascitic fluid by use of point of care dipstick. ***Material/method:*** A total of 67 patients with ascites were recruited for this study. Using serum ascites albumin gradient (SAAG) as standard way of classifying ascitic fluid into exudate and transudate, dipstick protein levels were assessed to classify the ascitic fluid into exudate and transudate. Ascitic fluid was obtained by abdominal paracentesis at the same time of collecting venous blood from the patients. SAAG was calculated based on albumin values of venous blood and ascitic fluid (SAAG less than 11g/L represented exudate while SAAG greater than 11g/L represented transudate. Values obtained were classified as high protein dipstick and low protein dipstick. ***RESULTS:*** Of the 67 adult population in this study,38 of them(56.7% were female, while 29(43.2%) were males($p<.005$).The age range was 18-65years with mean age of 47.11±11.21 for both sexes(male:50.8±9.4, female:44.29±11.78, p value of .0168). Mean body mass index(BMI) was 24.19±2.9.A total of 41(61.2%) had high ascitic fluid protein based on dipstick(500mg/dL),while 26 had low ascitic fluid protein(30mg/dL).SAAG was the gold standard used to differentiate exudates from transudate. In comparing dipstick protein(high or low)to correlate with low SAAG(exudates) and high SAAG(transudate) yielded a sensitivity,specificity,PPV,NPV and accuracy of 63.4%,42.3%,63.4%,42.3% and 70.3% respectively. ***CONCLUSION****:* SAAG is a better indicator of classifying ascitic fluid into exudate or transudate than urine dipstick. ***Keywords:*** *Dipstick, point of care, ascites, exudate, transudate, serum ascites albumin gradient.*

A-010

ORAL SUPPLEMENTS ASSOCIATION WITH DIETARY BENZO{A}PYRENE IN PROSTATE CANCER IN IBADAN,NIGERIA.

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Introduction
Prostate cancer has been linked to dietary factors. The presence of Benzo{a} pyrene; a prototype of Poly Aromatic Hydrocarbon (PAH) in foods is linked with various methods of food preparation. The relationship between dietary factors and the level of the toxicant- PAH in men with prostate cancer in Ibadan therefore needs to be established.
Methods
The study included thirty (30) prostate cancer patients aged 55-85 and thirty apparently healthy controls. Prostate Specific Antigen (PSA), Benzo {a} pyrene (BaP) and Total antioxidant Status (TAS) were analyzed using standard methods. BaP was analyzed using high performance liquid chromatographic technique (HPLC). Data obtained were analyzed statistically as appropriate and $p < 0.05$ was considered significant.
Results
The mean value of PSA was predictably significantly higher in Prostate cancer patients compared to the controls while, surprisingly there was a significant decrease in the level of BaP in men with prostate cancer compared with the controls. Correlation analysis however showed a significant positive correlation between smoked fish intake and BaP in the patients. TAS was significantly higher in the controls than in prostate cancer patients. Increased consumption of smoked foods, reduced TAS and resultant oxidative stress may contribute to the aetio-pathogenesis of prostate cancer. Antioxidant supplementation may enhance the total antioxidant status and may be of help in the management of prostate cancer.
Keywords: Prostate cancer; BaP, TAS, PSA

A-011

Establishment and performance evaluation of serum HE4 detected by chemiluminescence.

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Background: We intend to find a CLIA (Chemiluminescence immunoassay) method with good repeatbility, high sensitivity, wide linearity range to detect the serum HE4 rapidly, and then set up the performance. **Methods:** To establish adouble antibody sandwich CLIA method to detect the serum HE4. and evaluate the analytical performance including sensitivity, precision, accuracy, linearity, specificity under the optimized condition; we alsocompare the analytical performance and correlation with the commerical HE4 EIA test kit; Test clinical samples, then calculate the sensitiv-

ity, specificity and area under ROC (Receiver operating characteristic curve). **Results:**The precision of with-in lot and with-out lot are less than 4.0% and 5.0% respectively; Serum recovery is within 90.0% -110.0%; LoB is 5.0pmol/L;functional sensitivity is 15.0 pmol/L; measure range is 15.0~1500.0pmol/L; report range is 15.0~7443.0pmo/L; the R^2 is bigger than 0.95 comparing with commercial EIA test kit according to EP9A2; the diagnostic sensitivity is 79.5%, specificity is 92.0%, AUC(area under curve) is 0.908 (95% CI 0.865~0.951). **Conclusion:** we have established a sensitive and rapid CLIA method with highrepeatability and wide measurerange; it has good correlation with commercial EIA HE test kit, and there is no statistics difference on clinical diagnosis.

A-012

Profiling Of 5-hydroxymethylcytosine From Liquid Biopsy Samples: A Novel Approach For Early Diagnosis And Monitoring Of Cancer

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Background: The epigenetic modification 5-hydroxymethylcytosine (5hmC) plays a pivotal role in gene expression and has been associated with many diseases, including cancer. Unlike the relatively static genomic sequence, epigenetic DNA modifications are highly dynamic and change in response to both environment and disease status. Further, DNA modifications have been shown to occur early in disease development and exhibit tissue-specific signatures — making them exceptionally well-suited to the diagnosis and monitoring of disease using cell-free DNA (cfDNA). This study aims to assess the utility of a new, highly-sensitive 5hmC discovery platform to identify cancer-specific signatures from minimally invasive, liquid biopsy (LQB) samples. **Methods:** We have developed a novel 5hmC-pulldown technique (HMCP) that allows genome-wide profiling 5hmC distribution using low ng quantities of input DNA such as are typically recovered in cfDNA samples. Starting with 1ml of plasma collected from a cohort of healthy individuals and patients with different stages of cancer, we isolated cfDNA and processed these samples through the HMCP workflow prior to NGS analysis using specialised informatics algorithms. **Results:** We successfully identified 5hmC features that could differentiate colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) cases from controls and early from late-stage disease. These results indicate that this novel and sensitive method of 5hmC profiling holds the potential to identify novel disease- and tissue-specific biomarkers for the development of minimally invasive diagnostic and prognostic assays. **Conclusion:** 5hmC is a dynamic epigenetic mark that can provide valuable information for disease diagnosis and monitoring. The HMCP workflow developed by Cambridge Epigenetix is a robust, reliable, affordable and highly sensitive method to reveal the true hmC profile of DNA from different clinical conditions. By analysing cfDNA-derived hmC profiles from cancer patients and healthy individuals we have demonstrated that this could form the basis of a useful prognostic marker, especially for early stage cancer diagnosis.

A-013

Lack of Harmonization in Diverse Analytical Methods for Measuring Tumor Markers; CEA and CA19-9

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Background: Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) are widely used tumor markers for cancer screening and therapeutic monitoring. However, different results obtained for the same sample by using diverse analytical methods that are not harmonized can lead misinterpretation of the results and inappropriate clinical decision making. In this study, we performed a method comparability test for CEA and CA 19-9 near the claimed cutoff values by using widely used analyzers. **Methods:** Total of 103 residual samples for the CEA test and 101 samples for the CA19-9 test near-cutoff values, were collected from patients who have visited Seoul national university hospital (SNUH) and undergone health checkup or therapeutic monitoring from May 2016 to Jul 2016. Samples were stored at -70℃ and measured by four immunoassay analyzers, Architect i2000 (Abbott Laboratories, Abbott Park, IL, USA), Moduler E170 (Roche Diagnostics, Mannheim, Germany), Unicel DxI 800 (Beckman Coulter, Fullerton, CA, USA), ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), and immunoradiometric assay (IRMA). The results of each sample were collected and analyzed via method comparability test based on the CLSI EP9-A2 guideline, using Microsoft Excel (Microsoft, Redmond, WA, USA), IBM SPSS (SPSS Inc., Chicago, IL, USA), and EP evaluator (Data Innovations, South Burlington, VT, USA). Correlation between positive test results and clinical progress identified by medical record review was also analyzed using chi-square test. **Results:** Method comparison tests, comparing Architect the main instrument used in SNUH with other analyzers, were poorly correlated; results of quantitative measurement for CEA and CA19-9 were not clinically equivalent. We observed concordant qualitative test results in CEA measurement determined by the claimed cut-off values, between Architect and other analyzers (Moduler E170, $p < 0.001$; Unicel DxI 800, $p < 0.001$; ADVIA Centaur XP, $p = 0.001$) except IRMA ($p = 0.232$), and concordant test results in CA19-9 measurement were observed for all assays including IRMA ($p < 0.001$). However, we observed considerable disagreement, from 14 to 76 % for CEA, and from 13 to 35 % for CA19-9. There was no significant correlation between clinical disease progression in patients with cancer and the positive test results for CEA obtained from each assay (Architect, $p = 0.868$; Moduler E170, $p = 0.873$; Unicel DxI, $p = 0.204$; ADVIA Centaur XP, $p = 0.817$). Conversely, positive test results for CA19-9, obtained by using Moduler E170 and Unicel DxI showed statistically significant correlation with progressive disease ($p = 0.012$; $p = 0.028$, respectively). **Conclusion:** Considerable differences between analyzers in CEA and CA 19-9 assays were observed near-cutoff values; this discrepancy can lead to confusion in clinical decision making. The difference was greatest between the chemiluminescence immunoassay and immunoradiometric assay. Careful interpretation of test results with near-cutoff values and harmonization of different immunoassays, need to be mandatory.

A-014

Multicenter Comparison of Automated Immunoassay Analyzers for Prostate Specific Antigen

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Objectives: A multicenter study were performed to compare results of prostate specific antigen (PSA) using four different automated immunoassays (Immulite 2000 XPi, Unicel DxI800 Access, ADVIA Centaur XPT, and ARCHITECT i2000SR). **Methods:** One hundred and twenty serum samples collected on patients with PSA values between 0.00 and 104.37 ng/mL were assayed by the four reagents and analyzers. Wilcoxon test, Spearman's correlation, and Bland-Altman plot analysis were performed. **Results:** Among the 4 automated immunoassays, Immulite 2000 XPi showed the lowest median and mean value of PSA. The Unicel DXI 800 revealed the highest median and mean value. The significant differences in the results of PSA were observed in the comparison of each 4 instruments. However, the range of the correlation coefficients (r) with 4 instruments was between 0.991 to 0.995. Compared to Immulite 2000 XPi Immunoassay, the mean bias was less than + 0.816 ng/mL in other 3 instruments. Compared to Unicel, the mean bias was -0.767, -0.687, -0.816 ng/mL (vs ADVIA Centaur, Architect, and Immulite, respectively). In case of Architect, the mean bias was less than ± 0.687 ng/mL. The comparison of ADVIA Centaur and other 3 instruments, the mean bias was less than ± 0.767 ng/mL. The total agreements at the clinically relevant cut-off were optimal: between 95-99% at 4.0 ng/mL. **Conclusions:** The results of the automated four immunoassay analyzers comparison studies for PSA were acceptable. We evaluated this study by comparing 4 automated analyzers. Although Immulite 2000 XPi displayed a tendency of lower results under especially the clinical relevant cut off compared to other 3 analyzers, it yields high correlation coefficients and agreement at cut off. The results of the automated four 4 immunoassay analyzers comparison studies for PSA were acceptable and applicable at the clinically relevant cut-off.

Table 1. Comparison of PSA results obtained with 4 automated immunoassays

	Wilcoxon test (p)	Spearman's correlation (r)	Mean bias and 95% CI (ng/ml)	Agreement[a]
Immulute 2000 Xpi vs UniCel DXI 800	<0.0001	0.992 (p<0.0001)	0.816 (0.037 to 1.596)	95% (K, 0.92)
Immulute 2000 Xpi vs ADVIA Centaur XPT	0.0253	0.991 (p<0.0001)	0.049 (-0.366 to 0.464)	99% (K, 0.96)
Unicel DXI 800 vs ADVIA Centaur XPT	0.0004	0.995 (p<0.0001)	-0.767 (-1.395 to -0.140)	95% (K, 0.92)
Unicel DXI 800 vs ARICHITECT I2000SR	<0.0001	0.995 (p<0.0001)	-0.687 (-1.533 to 0.159)	98% (K, 0.96)
ARICHITECT I2000SR vs ADVIA Centaur XPT	0.0073	0.994 (p<0.0001)	-0.080 (-0.418 to 0.259)	98% (K, 0.96)
ARICHITECT I2000SR vs Immulute 2000 Xpi	0.0259	0.993 (p<0.0001)	-0.129 (-0.508 to 0.250)	98% (K, 0.96)

A-015

Development of a Versatile Platform for Routine Flourescence in-situ Hybridization

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Background: Fluorescence microscopy is a highly sensitive and valuable method in clinical diagnostics. Genetic aberrations in cancer biopsies are routinely observed with fluorescence in-situ hybridization (FISH). The increasing number of tumor patients, the high diversity of probes and the diagnostic effort for the correct interpretation of FISH signals, lead to the need for the development of an automated reading and evaluation platform. In fact, challenging parts are the heterogeneity and complexity of the tumor derived section. Thus, pre-filtering of tumor derived areas of interest, the detailed focus of these parts with subsequent probe detection and evaluation is required. **Methods and Results:** ***Simultaneous real-time device control*** The platform is based on a motorized microscope with changeable magnification and fluorescence filters, a LED illumination with multiple wavelengths, a precise moveable X-Y stage and a high-resolution greyscale camera[1]. All devices are controlled simultaneously by generating the appropriate driver software developed in house[2]. ***Pre-filtering*** Stitching strategy acquires images of DAPI (4,6-Diamidino-2-penylindole) stained specimens with low magnification (100-200 fold) getting a tissue overview within 30 min. Specific algorithms like the entropy filter remove background and artefacts within 1 min and search dense cell areas that are interesting for the detailed FISH probe analysis. Letter requires higher magnification (400-600 fold). Within these images, single cells are detected and separated with an adjustable watershed transformation algorithm. ***FISH signal analysis*** Detection and interpretation algorithms are adapted to the specific probes (ZytoVision GmbH), which focus the break-apart and translocation of genes and further events like the gain or loss of sequences. Further, recording sharp images of relevant nuclei in a five stack z-layer image gallery with 500 nm distances combined with a maximum intensity projection is necessary for diagnostic documentation. ***End-user evaluation*** Results are demonstrated within a user friendly graphical interface, performed as a diagnostic learning tool. Further, a long term archiving supports the routine workflow. **Conclusion:** The platform is an early stage of development and will be optimized for image processing and the self-learning program by using adapted algorithms. Further, routine diagnostics requires further optimization of scan time and image processing algorithm for multiple cancer sections. **References:** (1) A. Willitzki *et al.* (2012). Clin. Dev. Immunol. 284740. doi:10.1155/2012/284740.; (2) S. Rödiger *et al.* (2013). Adv. Biochem. Eng. Biotechnol. 133 35-74. doi:10.1007/10_2011_132.

A-016

Incidence of Antigen Excess Utilising the Freelite® Assays on the Optilite® Analyser

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Introduction

Serum immunoglobulin free light chain (FLC) concentrations can range from <1mg/L up to >10,000mg/L and therefore, any immunoassay measuring serum FLCs has a risk of underestimation due to antigen excess. The Optilite analyser is able to detect samples in antigen excess through a suite of methods which include reaction kinetic monitoring and the addition of patient sample post-reaction. For both κ and λ Freelite assays, reaction kinetics are monitored over three separate windows; a change in rate of reaction between the windows is indicative of potential antigen excess. Samples with such kinetics are flagged and automatically re-run at a higher dilution to preclude a falsely low result. Here we analyse data collected from four evaluation laboratories between January 2015 and September 2016 to determine the efficacy of the antigen excess check for the Optilite Freelite assays.
Method
We reviewed κ and λ FLC results generated using Freelite assays (The Binding Site Group Ltd., Birmingham, UK) on an Optilite analyser (The Binding Site Group Ltd., Birmingham, UK) at four evaluation laboratories. 10495 individual sample results were analysed using κ Freelite and 10228 using λ Freelite. Results were categorised into the dilution at which they were obtained and the frequency of antigen excess flags at each dilution was reported. The robustness of the antigen excess check was established by comparing each flagged sample result to the result obtained at the next highest dilution. If a higher result was produced at the next dilution, the original antigen excess flag was determined to be correct. Conversely, if a lower result was obtained, the antigen excess flag was deemed incorrect.
Results
Overall, 11% (971/8573) of samples were flagged for antigen excess using the κ Freelite assay at the standard sample dilution of 1/10, 10% (156/1518) at the reflex high dilution of 1/100 and 7% (18/266) at the further reflex high dilution of 1/1000. For the λ Freelite assay, 6% (512/8536) of samples were flagged for antigen excess at the standard sample dilution of 1/8, 11% (87/795) at the reflex high sample dilution of 1/80 and 5% (7/134) at the further reflex high dilution of 1/800. The frequency of correct antigen excess flags was ≥95.6% for κ Freelite and ≥98.9% for λ Freelite at each sample dilution. Since March 2016 there have been no examples of undetected antigen excess reported.
Conclusions
Of over 10,000 sample results, 98% (1121/1145) of samples were flagged correctly for antigen excess using κ Freelite assays whilst 99% (601/606) of samples were flagged correctly for antigen excess using λ Freelite assays. This validates the ability of the analyser to accurately flag and re-dilute samples in antigen excess and demonstrates that the occurrence of undetected antigen excess with these assays is negligible.

A-017

Laboratory performance of serum free light chain immunoassays in baseline and post-treatment samples of light chain multiple myeloma patients

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Background: Serum free light chain (sFLC) measurements are routinely used in the diagnosis and management of patients with monoclonal gammopathies. Assessment of sFLCs is particularly informative in light chain multiple myeloma (LCMM) patients; an abnormal sFLC ratio is expected in all cases at diagnosis and monoclonal (involved; iFLC) levels provide a sensitive marker of response. This patient group can present a particular challenge for FLC measurements since high iFLC levels are often present at diagnosis, requiring the analyser to perform additional sample re-dilutions until a final result is reported. However, novel treatments for MM can lead to rapid reductions in the concentration of monoclonal FLCs in serum. Here we compare the number of Freelite® re-dilutions required following initial treatment of LCMM patients compared to baseline samples, within the context of a clinical trial. **Methods:** Serum samples from 113 LCMM patients enrolled on to the IFM 2009-01 study were assessed for κ and λ FLC concentrations using Freelite immunoassays (The Binding Site Group Ltd, UK) on the BN™II nephelometer (Siemens, Germany). Samples were analysed at baseline, and following 1 and 3 cycles of induction therapy with bortezomib, lenalidomide and dexamethasone. Sample re-dilutions needed for achieving a final iFLC result was compared at each time-point. **Results:** At baseline, all 113 patients had an abnormal κ/λ FLC ratio (72 κ and 41 λ). The mean iFLC was 3798.2, 613.8 and 204.0 mg/L for κ FLC, and 3402.1, 395.7 and 125.0 mg/L for λ FLC at baseline, cycle 1 and cycle 3, respectively. For κ iFLC measurements, 11% of samples gave a final result on the standard dilution at baseline, compared to 31% after cycle 1 and 54% after cycle 3. For λ iFLC measurements, 5% of samples gave a final result on the standard dilution at baseline, compared to 54% after cycle 1 and 63% after cycle 3. The average number of re-dilutions required at baseline to achieve a final result per patient was 1.9 for both κ and λ iFLC.

After 1 treatment cycle the average number of re-dilutions required for achieving a final result was 0.8 for κ iFLC and 0.7 for λ iFLC. The average number of re-dilutions required at cycle 3 was lower than at cycle 1 for both κ (0.5) and λ (0.4). **Conclusion:**Novel myeloma drugs lead to rapid reductions of monoclonal sFLC levels in LCMM patients, impacting the number of sample re-dilutions required by Freelite immunoassays for reporting a final result on the BNII instrument. Laboratories should be reassured by the reduction in the need for re-dilutions in individual MM patients after initiating treatment.

A-018

Development and Testing of Reference Materials for NGS based Somatic Variant Detection and Fusion Detection in Myeloid Cancers

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Background: Myeloid malignancies are clonal diseases of hematopoietic progenitor cells which can lead to accumulation of immature blast cells in the bone marrow and peripheral blood. Understanding the molecular changes that lead to the clonal proliferation can aid in both determining prognosis and therapy selection. The large range of genetic aberrations and genes involved make Next Generation Sequencing (NGS) tests a cost effective and sensitive way to determine these molecular changes. There are an increasing number of NGS-based oncology tests available for somatic mutation detection in patients with hematological malignancies. However, reference materials that contain mutations relevant to blood cancers are lacking. **Methods:** We designed a reference material in purified RNA format that contains nine RNA fusions important in myeloid cancers including two different ETV6-ABL1 transcripts, as well as BCR-ABL1, MYST3-CREBBP, RUNX1-RUNX1T1, and PML-RARα among others. We designed a second reference material in purified DNA format to include 23 somatic variants included two FLT3 internal tandem duplications. The biosynthetic RNA or DNA constructs were mixed with either total RNA or purified genomic DNA from GM24385 reference human cell line. Digital PCR was used to quantify the variant sequences to determine the abundance of the fusion RNAs and the allele frequencies of the somatic mutations. Testing was then performed on a variety of targeted NGS assays to show compatibility. **Results:** Testing of the Seraseq™ Myeloid RNA Mix by Digital PCR confirmed that each of the nine fusions in the reference samples are present at approximately 100 fusion copies per nanogram of total RNA. FusionPlex Myeloid Kit for Illumina (ArcherDx) as well as Oncomine Myeloid Panel (Thermo Fisher) showed positive detection for all fusions present in the reference samples. Seraseq™ Myeloid DNA Mix was also tested by digital PCR and results confirmed the variant allele frequencies were on target at the 5%, 10% or 15% VAF. Effective dPCR allele specific assays could not be obtained for CEBPA mutations. However, NGS testing of the reference samples by VariantPlex Core Myeloid Kit for Illumina (ArcherDx) and Oncomine Myeloid Panel (Thermo Fisher) confirmed the presence of these mutations at the expected levels, as well as all of the other variants in the DNA reference samples. **Conclusion:** SeraCare has developed highly multiplexed DNA and RNA-based reference materials for evaluating Myelogenous disorders that allow monitoring of a broad range of somatic mutations and gene fusions. These reference materials aid optimization and verification of detection limits for NGS-based Myeloid disease assay testing, and provide laboratories greater assurance in their ability to correctly detect and quantify various types of genetic events in diseased patient samples.

A-019

Potential Serum Metabolic Markers in Thyroid Carcinoma Diagnosis

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Background: Distinguishing between the benign and malignant types of thyroid nodules is still challenging in clinical laboratories. Since these two types of tumors require totally different interventions in clinic, it is necessary to establish new methods for accurate diagnosis of thyroid cancer. Methods: We use Nuclear magnetic resonance spectroscopy (mostly ^{1}H-NMR) -based metabolomics approach to analyze the metabonome of human serum from 33 thyroid cancer (TC) patients including 20 Papillary thyroid cancer (PTC) patients and 13 Medullary thyroid cancer (MTC) patients, 17 nodular goiter (NG) patients and 14 healthy controls (HC) from the Peking Union Medical College Hospital (PUMCH) between 2013 August and 2014 December. The unique feature of this study was the combination of metabolomics and classification tree analysis in order to improve the discrimination with the least error. Results: Compared with NG group and HC group, TC group has increased glucose, creatine, betaine, sucrose, L-serine, L-valine, phenylalanine and methionine levels and decreased concentration of fructose, tyrosine, aspartate, L-lactic acid, glycine, glutamic acid, guanosine, β-alanine, histidine, L-glutamine, L-alanine and the receiver operating curve (ROC) shows good sensitivity and specificity in classifying cases. Betaine, D-glucose, L-alaine and Glucose stand out with the area under roc curve (AUC) greater than 0.850. Among those four chemicals, D-glucose shows an excellent performance in sensitivity and specificity (both 0.9) and the AUC is 0.888. Further analysis shows relative high serum Lactate, L-proline and sucrose levels and lower levels of aspartate, betaine, D-glucose, L-alanine and maltose in PTC group when compared to MTC group. Conclusion: Our study illustrates that the NMR-based metabolomics approach was shown to investigate the possible serum biomarkers for thyroid cancers. We are aware that metabolomics profile of serum sample extract may be altered and released from the original cancer cells. Serum can also be used to construct a predictive model with high sensitivity and specificity for thyroid cancer as less invasive but more acceptable sample type.

A-020

Development and Validation of ColoScape - A New Colorectal Cancer Mutation Detection Assay

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Background: Colorectal cancer is a highly preventable disease. Herein we report the development and validation of ColoScape, a novel multigene mutation biomarker real-time PCR based assay for qualitative detection of colorectal cancer associated biomarkers in the following genes: *APC (Exon 15), KRAS (Exon 2), BRAF (Exon 15) and CTNNB1 (Exon 3)*. **Methods:** The high sensitivity of ColoScape is achieved by xenonucleic acid (XNA) probe technology. XNA probes are backbone modified oligomers with natural nucleoside bases (A,T,C and G) that hybridize by Watson-Crick base pairing to natural DNA and RNA with higher binding affinity. XNA probes bind to the selected wild-type sequences at the respective genetic loci in the target genes. For selected mutation sites, primers and FAM-labeled TaqMan probes were designed and tested with the selected XNA oligomers. An internal PCR control selected in the Human b-Actin (ACTB) gene was employed utilizing a HEX-labeled TaqMan probe. Performance parameters were established on colorectal cancer patients DNA extracted from FFPE and reference DNA materials. **Results:** At least 0.5% mutation in wild-type background can be detected by Coloscape for target mutations in APC (exon 15), CTNNB1(exon 3), KRAS (exon 2) and BRAF V600 (exon15) with APC c1450 and CTNNB1 assays showing sensitivity of < 0.1% mutation in 5-10 ng of WT DNA/well. No cross reactivity was observed with wild-type up to 320ng purified gDNA and up to 20ng FFPE DNA per reaction demonstrating high specificity of the ColoScape. Intra-assay, inter-assay, lot-to-lot and operator variation comparison showed CV% between 3% and 8%. Excluding pre-cancer samples, the assay clinical specificity and sensitivity were 95% and 100%, respectively. Pre-cancer detection sensitivity was 60% (6 out of 10 FFPE samples) and 62.5% for stool samples. For tested FFPE clinical samples, the assay specificity and sensitivity were 95% and 91% respectively while the assay clinical specificity and sensitivity were both 100% for plasma samples. **Conclusions:** The ColoScape Colorectal Cancer Mutation Detection assay is a sensitive tool intended to facilitate research in colon cancer development, early detection, disease monitoring and therapeutic interventions.

A-021

EGFR mutation detected in cfDNA from cerebrospinal fluid permit the diagnosis of leptomeningeal metastases in a patient with lung adenocarcinoma

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Background: EGFR activation mutations predict susceptibility to tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer (NSCLC). Although patients render

positive response to TKIs initially, resistance could eventually develop due to the secondary mutation T790M. Multiple resistant subclones can arise following treatment of NSCLC in patients with EGFR-targeted therapies. Therefore, the degree of heterogeneous distribution of these mutations regarding the metastasis localization may be of importance in order to a better tailored treatment. Leptomeningeal metastasis is an uncommon complication in patients with solid tumors and appears to be more frequent in lung cancer patients harboring EGFR mutations. Liquid biopsies using plasma-derived cell free DNA (cfDNA) are non-invasive tests that allow a better selection and monitoring of the patients with NSCLC. cfDNA can be found in serum, plasma or urine. However, there exists little evidence of cfDNA detection in other body fluids, where the methodology is not optimized yet. **Case report:** A 63-year-old man was admitted to the hospital for an episode of oppressive frontal headache that lasted ten days, not responding to treatment. 15 months before, he had been diagnosed with stage IV pulmonary adenocarcinoma by tissue biopsy, showing EGFR mutated with deletion in exon 19. Since the beginning of this diagnosis afatinib was administered. A new tissue biopsy assay, performed two months before the headache event, showed the additional acquisition of the T790M resistance mutation in EGFR, with a change in the pharmacological therapy to osimertinib. MRI performed on admission showed neither space occupying lesions nor suggestive signs of metastatic. Small lesions with a pattern suggestive of involvement of small vessels were described and a CSF study was requested. CSF biochemical results were: Proteins, 54.10 mg/dL (15.00-45.00 mg/dL); glucose, 57.60 mg/dL (plasma glucose, 73.20 mg/dL); Chlorine, 118.20 mEq/L (120-130 mEq/L). The CSF was transparent in appearance and the cellular recount was 28 cells/mm3. The cells were stained using a rapid panoptic and observed at 1000x magnification. They were of bigger size than leukocytes and showed an atypical appearance, with large and rounded nucleus. They were reported as possible tumor-derived cells, suggesting leptomeningeal carcinomatosis secondary to pulmonary adenocarcinoma. Subsequently, a cell free DNA (cfDNA) test for EGFR mutations by Real Time qPCR was performed on the CSF supernatant. It resulted in the detection of EGFR deletion of exon 19. We did not detect the T790M resistance mutation in the CSF sample, althought this mutation did was demonstrated in conjunction with the deletion of exon 19 in a subsequent analysis of cfDNA in plasma. **Conclusions:** The microscopic analysis of CSF performed in the emergency laboratory oriented the clinical diagnosis towards leptomeningeal dissemination of adenocarcinoma cells. The cfDNA study of EGFR was adapted for the detection of EGFR mutated in CSF, where a positive result confirmed the malignant origin of the cells. The presence of EGFR exon 19 deletion and the absence of the T790M resistance mutation in CSF, show the existence of tumor heterogeneity, revealing in this patient possible differences in the metastatic spread or a differential site-dependent pharmacological response of both clones of cells.

A-022

ASSOCIATION BETWEEN HE4 AND COLORECTAL CANCER

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Background: Human Epididymis Protein 4 (HE4) is a secretory protein originally identified in the distal human epididymis. Serum levels of HE4 have been investigated in patients with ovarian and endometrial cancer but only a few have described the role of HE4 in colorectal cancer. **Methods:** One hundred and twelve healthy individuals (47 men and 65 women) were recruited. Eighty seven patients with a diagnosis of colorectal cancer were selected (50 men and 37 women). No other pathology was found in this group. Ca19.9 and CEA were performed using ADVIA Centaur XP System (Siemens®). HE4 was analyzed in the Cobas Elecsys E411 (Roche®). Statistical analysis was performed with SPSS. Marker comparison test between case and control groups were made using U of Mann-Whitney. Student's t-test was made for Age comparison and Chi-squared for gender comparison. **Results:** HE4 median in case group was 85.67 (47.23) pmol/L. HE4 median in control group was 52.19 (19.51) pmol/L. Ca19,9 median in case group was 18.00 (73.50) U/ml and 9.23(11.00) U/mL in control group. CEA median in case group was 2.80(7.00) ng/mL and 0.76(0.93) ng/mL in control group. We found a statistically significant differences in HE4 adjusting by age ($p<0.001$), Ca19.9 and CEA showed an adjusted by age significant of $p=0.012$ and $p=0.005$ respectively. HE4 sensitivity and specificity was calculated with a result of 82.8% and 82.3% respectively with a cutoff point of 63.30 pmol/L. **Conclusion:** The present study suggests a positive association between HE4 levels and colorectal cancer which is stronger than Ca19.9 and CEA. Further studies are needed with a longitudinal design to investigate the value of HE4 as a biomarker in this pathology.

A-023

Role of β human chorionic gonadotropin, α-fetoprotein and lactate dehydrogenase for testicular germ cell tumors in a sample in the city of Sao Paulo.

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Background: Testicular tumor is the most common cancer in men and can occur at any age. Among the most common types of germ cell tumors (GCT) we have: Seminomatous Tumor that is a type of testicular cancer originating in the germinal epithelium of the seminiferous tubules. The Nonseminomatous, where we find the Embryonic carcinomas that are highly malignant, with rapid growth and with early metastases, while the Choriocarcinomas are the rarest and most aggressive. Finally, we have the Teratomas that are formed by one or more tissues embryonic. Most non-seminomatous tumors are mixed with at least two types. The objective of this study was to study the behavior of the tumor markers of GCT before the pathological anatomy tests of testicular biopsy.METHODS-This was a retrospective, observational study in which 4943 β-HCG positive for the period from 2015 to 2017 were analyzed in male patients from a laboratory in Sao Paulo. Of these, we performed 328 positive b-HCG tests with AFP and DLH for GCT. We have focused on 30 tests with testicular biopsies performed together with the serum tumor markers in the same laboratory. For the dosages of β-HCG and AFP, we use the equipment ARCHITECT i2000SR Abbot® and UNICEL DXI Beckman&Counter . DHL through the equipment AU 5842 Beckman&Counter. Pathological anatomy is analyzed by optical microscopy. Immunohistochemistry through the Autostainer Link 48 Agilent and optical microscopy. RESULTS- The findings of the 30 biopsies and β-HCG positive for testicular cancer were shown in Table 1. CONCLUSION-Serum tumor markers studied for GCT showed great relevance for identification of tumor origins, being essential for stratification of testicular neoplasm, including the classic treatment of orchiectomy (removal of the testis).The classic seminoma, a germ cell tumor with higher incidence, showed a significant alteration in 50% of the cases of β-HCG dosages and there was no cases of AFP changes.

Table 1: Differences of testicular cancer and the relation regarding the alteration of tumor markers β-HCG, AFP and DHLin n=30 cases:

		βHCG+	DHL+	AFP+
N=30	n	%	%	%
Embryonic Carcinoma	8	100	100	71.4
Choriocarcinoma	1	100	/	100
Teratoma	1	100	100	100
Non-Seminomatous	1	100	100	100
Classic seminoma	10	100	77.7	0
Mixed GCT	9	100	80	87.5

A-024

Expression of Tripartite motif family protein72 (TRIM72) in tumor tissue and serum of patients with colon cancer and its clinical significance

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Background: Colon cancer is one of the most common malignancies worldwide, which causes a major population of cancer-related deaths in the world. The tripartite motif family protein 72 (TRIM72), also known as MG53, is involved in the insulin resistance and metabolic syndrome which are the risk factor of colon cancer. However, the expression of TRIM72 in the colon cancer tissues and its serum level in colon cancer patients still remain unknown. **Methods:** We investigated the expression patterns of TRIM72 in colon cancer tissues and normal tissues by immunohistochemical staining. The serum level of TRIM72 was also measured using ELISA kit. The receiver operating characteristic curve (ROC) curves were applied to evaluate the diagnostic value of TRIM72 level for colon cancer patients. **Results:** The results showed that the expression level of TRIM72 in colon cancer tissues and its serum level in colon cancer patients were significantly decreased compared with normal controls. Additionally, low level of TRIM72 expression was associated with advanced clinical stage, the lymph node and distant metastasis in colon cancer patients. Moreover, the ROC analytic results showed that TRIM72 has a better diagnostic value (AUC = 0.829) than the traditional tumor biomarker Carcinoembryonic Antigen (CEA) (AUC = 0.707) or Carbohydrate Antigen 19-9 (CA199) (AUC = 0.750); and the combination of TRIM72 with CEA and CA199 showed the best diagnostic value for colon cancer (AUC = 0.928).

Conclusion: These results suggested that TRIM72 may have the potential to be a new biomarker for the diagnosis of colon cancer.

A-025

Plasma D-dimer level improves prediction of distant organ metastasis in colorectal cancer

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide, and its mortality rate continues to increase yearly in less developed regions. Distant organ metastasis is mainly responsible for the high rate of death associated with CRC. Currently, the diagnosis of distant organ metastasis of CRC mainly relies on pathological examination, imaging techniques and serum tumor biomarkers, but each has its own limitations. D-dimer is a degradation product of cross-linked fibrin, and plasma D-dimer levels have been demonstrated to be correlated with disease stage and prognosis in CRC patients. However, few studies have focused on the predictive value of D-dimer for distant organ metastasis in patients with CRC. **Objective:** The aim of the study was to evaluate the diagnostic performance of D-dimer level in predicting distant organ metastasis in CRC. The results were then compared with those of serum serum carcinoembryonic antige (CEA) levels, to determine whether D-dimer could improve the predictive value of CEA for distant organ metastasis. **Methods:** 106 CRC patients with distant organ metastasis and 86 ones without distant organ metastasis were included in the study. All patients were histologically diagnosed either by endoscopic or surgical specimen examination. The identification of metastatic lesion(s) was based on the results of imaging examination, image-guided biopsy or exploratory laparotomy. Plasma D-dimer and CEA values were measured in these CRC patients. The diagnostic performance of D-dimer levels in predicting distant organ metastasis was examined by receiver operator characteristic (ROC) curves, and then was compared with the performance of CEA. **Results:** The median of D-dimer values in patients with distant organ metastasis was higher than the level for patients without distant organ metastasis (0.72 mg/L FEU versus 0.27 mg/L FEU, p<0.0001). The result was not affected by patient age, smoking history or previous treatment. D-dimer levels significantly correlated with CEA levels in patients with CRC, but D-dimer had a larger area under ROC curves (0.80) compared to CEA (0.75). Combined with D-dimer assay, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the CEA assay for predicting distant organ metastasis in CRC can be increased to 76.4%, 80.2%, 82.7%, 73.4% and 78.1%, respectively. **Conclusion:** Plasma D-dimer values can improve prediction for distant organ metastasis in CRC. Both D-dimer level and CEA level elevation are clinical indications for detailed imaging or pathological examination for distant organ metastasis in at risk patients.

A-026

Serum carboxylated osteocalcin test as a surrogate marker of bone metastasis in non-small cell lung cancer patients: impact of radiation/chemotherapy on confirmed bone metastasis

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Background: Early detection and active monitoring of bone metastasis (BMs) is important in lung cancer patients. In this retrospective study, we investigated the feasibility of using carboxylated osteocalcin (Gla-OC) as a surrogate marker of BMs and the impact of radiation/chemotherapy on the Gla-OC levels in patients with advanced lung cancer and confirmed BMs. **Methods:** Totally 283 patients with non-small cell lung cancer (NSCLC) were included in this study. Serum levels of Gla-OC was measured and compared between patients with BMs and those without, and patients with/without radiation/chemotherapy on confirmed BMs and lung cancer. Statistical analysis was performed using the SPSS software package (SPSS, Munich, Germany). **Results:** Among 283 advanced lung cancer patients with average age (59.1) including 170 male (60.1%) and 113 female (39.9%), Gla-OC in the 99 BMs patients was higher [Median (P25, P75):10.14 (7.02, 16.98) vs.9.75 (7.39, 12.43), P<0.01] than the group of 184 patients without BMs. In a subgroup of patients with lung squamous cell carcinoma (LSCC) without exposure to radiation/chemotherapy, Gla-OC was higher in the BMs group [13.40 (7.74,18.80) vs. 9.52 (6.55,11.76), P<0.05] than control group. Moreover, Gla-OC was lower [7.00 (6.07,11.26), n=9, P<0.05] in BMs LSCC patients exposed to radiation/chemotherapy with a poor detection sensitivity at 1.11%. In patients with lung adenocarcinoma (LAC), Gla-OC was higher in BMs group [12.13 (7.77,17.60) vs. 9.75(7.35,12.15), P<0.01] than the control group. Additionally, Gla-OC was lower [7.46(5.83,11.57), n=27, P<0.001] in BMs LCA patients exposed to radiation/chemotherapy. **Conclusion:** Carboxylated osteocalcin may be a viable surrogate marker of BMs in NSCLC patients who are not exposed to radiation/chemotherapy. Although Gla-OC in patients with radiation/chemotherapy exhibits poor sensitivity for BMs, it might be an indication of effective radiation/chemotherapy. More prospective studies are warranted to determine the Gla-OC in the evaluation of BMs and the impact of radiation/chemotherapy.

	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	Accuracy
All NSCLC	33	20	164	66	33.3%	89.1%	62.3%	71.3%	69.6%
Lung squamous cell carcinoma									
without radiation/chemotherapy*	10	3	20	10	50.0%	87.0%	76.9%	66.7%	69.8%
with radiation/chemotherapy**	1	2	16	8	1.1%	88.9%	33.3%	66.7%	63.0%
Lung adnocarcinoma									
without radiation/chemotherapy*	17	10	96	26	39.5%	90.1%	63.0%	78.7%	75.8%
with radiation/chemotherapy***	5	5	32	22	18.5%	86.5%	50.0%	59.3%	57.8%
*p<0.05 between BMs and control group; **P<0.01 between groups of BMs patients with and without radiation/chemotherapy; *** P<0.001 between groups of BMs patients with and without radiation/chemotherapy;									

A-027

TP53 mutations correlate immunohistochemical staining pattern of p53 and codon 72 polymorphism in mature T-cell and NK-cell lymphomas

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Background: : Mature T-cell and NK-cell lymphomas consist of a heterogeneous group of neoplasms with cytogenetic and molecular diversities. *TP53* mutation is known to be involved in the event of tumorigenesis and present in a variety of cancer subtypes. However, *TP53* mutation in T-cell and NK-cell lymphomas has less been reported. The aim of the study was to identify *TP53* mutation in different entities of mature T-cell and NK-cell lymphomas and further correlate with p53 expression. **Methods :** Fifty-seven cases of T-cell and NK-cell lymphomas obtained between 2006 and 2017 from the archives of the Department of Pathology, Chang Gung Memorial Hospital at Kaohsiung, Taiwan were collected for next-generation sequencing (NGS) and p53 immunohistochemical study. **Results:** By NGS, all samples showed *TP53* mutation with diverse mutation patterns and sites. All cases (100%) had missense variant of *TP53* mutation followed by stop gained variant (86.0%). Ten cases had mutation burden > 5% VAF, predominantly in extranodal nasal-type natural killer/T-cell lymphoma (NKTCL, 21.7%) and intestinal T-Cell Lymphoma (ITCL, 60%). No angioimmunoblastic T-cell lymphoma patients had *TP53* mutation with high mutation burden. Overexpression of p53 was observed in 11 (19.6%) of 56 tumors with variable extent. The percentage of tumor cells with strong p53 staining was positively correlated with *TP53* mutation VAF (R^2 = 0.95, P < 0.001). Furthermore, six (37.5%) of 16 cases with 72P homozygous genotype showed higher frequency of *TP53* mutation VAF >

5% as compared with 72R homozygotes (2/16, 12.5%) and heterozygotes (2/25, 8%) ($P = 0.04$). **Conclusions :** We demonstrated frequencies of *TP53* mutations in mature T-cell and NK-cell lymphomas with a higher mutation VAF identified in NKTCL and ITCL, and the p53 expression was positively correlated with *TP53* mutation VAF.

A-028

Methylation of NFBP1 as a novel marker for the detection ofplasma cell-free DNAinbreast cancerpatients

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Background: Circulating cell-free DNA (cfDNA) has been considered as a potential biomarker for non-invasive cancer detection, which is worthy of further validation and potentially benefits a broad range of applications in clinical oncology practice. Human Neuroblastoma Break Point Family, member 1 (NBPF1) are originally identified in a neuroblastoma (NB) patient .The present study was the first to determine the presence of NBPF1-methylated circulating cfDNA in plasma among breast cancer,benign breast disease and healthy control . Methods: Specific gene screening using bisulfite sequencing (BS-seq) among 25 consecutive breast cancer patients without treatment before surgery (stage I-III) and 25 benign breast diseases and 25 healthy female volunteers collected at Peking Union Medical College Hospital from 2016 to 2017. After that the three groups of 10 plasma samples were mixed to verify the methylation status of five sites for candidate gene NBPF1 in circulating cell-free DNA and further two sites of promoter for NBPF1 were detected in 50 breast cancer patients, 33 benign breast diseases and 30 healthy control by using methylation-specific PCR (MSP). Results: Breast cancer patients whose NBPF1 methylation levels are significantly higher than those of benign breast diseases and healthy controls at the same time are chosen as candidate gene. MSP result shows five sites for NBPF1 differ among the three groups in mixed samples. Methylation rate of two sites of the NBPF1 promoter were 63.1% and 66.7% of breast cancer patients (stage I-III) and 57.1% and 50.0% of benign breast disease, and 48.0% and 41.0% of healthy control respectively . One site methylation rate was significantly different among the three groups ($p<0.05$). Conclusions: These results indicate that NBPF1 promoter hypermethylation, which occurs in a significant proportion of breast tumors, and that NBPF1-methylated cfDNA thus may serve as a tumor marker for breast cancer in a large cohort of breast cancer patients. Large samples are still needed to verify the results, we will explore further.

A-029

Next generation sequencing identifies additional actionable markers of primary colon and lung adenocarcinomas in a south Florida veteran population.

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Background: Molecular profile testing of tumor tissues is a growing and vital need in the treatment of patients with colon cancer and non-small cell lung cancer. According to the National Comprehensive Cancer Network (NCCN) guidelines, routine molecular testing is recommended to identify rare driver mutations that may be present in tumor tissues. Proven responses to molecular therapies have greatly increased the improvement of care in colon and lung cancer patients. Currently, NCCN guidelines for colon adenocarcinomas suggest testing for tumor mismatch repair or microsatellite instability status and determination of tumor gene status for RAS (KRAS and NRAS) and BRAF. The current NCCN guidelines for adenocarcinomas in non-small cell lung carcinoma include testing for EGFR, ALK, ROS1, BRAF and PD-L1 expression. Present test methods for both sets of guidelines include FISH, PCR based sequencing, and immunohistochemistry. The objective of the study was to identify additional actionable markers from colon and lung adenocarcinomas, through next generation sequencing, that would not be detected based on current test methodology. **Method:** Eighteen cancer cases were reviewed and selected from male adenocarcinoma patients for next generation sequence testing. Samples were sent to Personal Genome Diagnostics (Baltimore, MD) for Cancer Select 125 testing. Next generation sequence testing was performed by analyzing the coding regions of 125 genes and identifying tumor-specific alterations in categories including: microsatellite instability, sequence mutations, amplifications, and rearrangements. The annotated reports included detailed analysis of mutations detected, FDA-approved therapies (for same and other indications) and current clinical trials. Actionable markers were identified and categorized based on sequence mutations, amplifications and rearrangements. **Results:** Actionable markers detected by next generation sequencing were divided based on colon versus lung primary tumors. Results were accumulated over a six-month testing period. Current molecular markers tested, according to NCCN guidelines, were removed from the data set leaving only the additional actionable markers. Microsatellite instability markers, KRAS, BRAF, and NRAS mutations were removed from the colon adenocarcinoma category. EGFR mutations, BRAF mutations, ALK and ROS1 rearrangements were removed from the lung adenocarcinoma category. The colon primary tumor category (N=14 patients) yielded 12 additional actionable sequence mutations, 9 amplifications and 5 microsatellite markers. The lung primary tumor category (N = 4 patients) yielded 9 additional sequence mutations, 5 amplifications and 1 rearrangement. **Conclusions:** Next generation sequencing of primary colon and lung adenocarcinomas provides accurate and comprehensive data detailing actionable gene alterations within a tumor sample. Tumor molecular alterations are listed in categories such as: consequence of sequence mutation, exon location, mutant fractions, and fold increases for amplifications. Gene alterations are further designated by approved current FDA approved therapy and current clinical trials. Our study identified several additional actionable markers within each sample that would not have been discovered utilizing our current test methods. Based on our findings, we conclude that next generation sequencing is an accurate and powerful test method that should be performed whenever adequate tumor tissue is available. This material is the result of work supported with resources and the use of facilities at the James A. Haley VA Hospital.

A-030

Performance Evaluation of the New Latex FLC kappa and lambda Assays on the Atellica® CH930 Analyzer

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Background: The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free light chain (FLC) determination as aid in diagnosis and management of clonal plasma cell disorders. We describe reproducibility, imprecision, method comparison, and limit of quantitation (LoQ) data for the application* of N Latex FLC kappa and lambda assays on the new Atellica® CH 930 Analyzer. **Methods:** Latex-enhanced mouse monoclonal antibody reagents from Siemens Healthineers for FLC kappa (N Latex FLC kappa) and lambda (N Latex FLC lambda) were assayed on the Atellica® CH 930 Analyzer. A precision study was conducted according to CLSI guideline EP05-A3 to estimate repeatability of four sample pools and two controls each for FLC kappa and lambda, in combination with one reagent and one calibrator lot. Each sample was assayed in quadruplicate twice a day for five days. A method comparison study was conducted according to CLSI guideline EP09-A3. The results were correlated with data generated on the BN ProSpec® System. The results were analyzed using Passing-Bablok linear regression analysis. LoQ was determined for both applications with one reagent lot following CLSI guideline EP17-A2. **Results:** The within-run CV for the new FLC kappa application on the Atellica® CH 930 Analyzer ranged from 0.80−2.09% and the total (within-instrument) CV from 1.50−3.78%. For FLC lambda, the within-run CV was 0.81-2.18%, and the total (within-instrument) CV was 1.76-4.75%. Passing-Bablok regression results between the BN ProSpec System and Atellica® CH 930 Analyzer were y = 1.008 x -0.064 mg/L (r = 0.986) for FLC kappa (n = 175) and y = 1.033 x -0.695 mg/L (r = 0.973) for FLC lambda (n = 168). LoQ was determined to be 1.084 mg/L for FLC kappa, showing a total error of 28.01%. LoQ for FLC lambda was 1.558 mg/L, with a total error of 15.76%. **Conclusion:** The new application of N Latex FLC assays on the Atellica® CH 930 Analyzer demonstrated acceptable and consistent imprecision. Method comparison results showed good agreement with an on-market assay. *Under development. Not available for sale.

A-031

Performance evaluation of serum PIVKA-II measurement using HISCL-5000 and method comparison of HISCL-5000, LUMIPULSE G1200 and ARCHITECT i2000

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Backgroud: Protein induced by vitamin K antagonist-II (PIVKA-II) is a useful tumor marker in addition to alpha-fetoprotein for the diagnosis of hepatocellular carcinoma (HCC). In this study, we evaluated the analytical performance of serum PIVKA-II measurement using HISCL-5000 analyzer (Sysmex Corporation, Kobe, Japan), and investigated its clinical usefulness in patients with HCC.

Methods: A total of 502 subjects (347 male and 155 female, median age 59.0 years) were enrolled. Among them, 335 were HCC patients, 45 were patients with non-HCC liver disease including liver cirrhosis, chronic hepatitis, HBV or HCV carrier, hepatic adenoma and intrahepatic cholangiocarcinoma, and 122 were healthy individuals. We evaluated the precision and linearity of PIVKA-II assays by HISCL-5000 analyzer. Method comparison was done among HISCL-5000, LUMIPULSE G1200 (Fujirebio Diagnostics, Fujirebio, Japan) and ARCHITECT i2000 (Abbott Diagnostics, Abbott, USA) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). **Results:** Repeatability (%CV) in low, high level controls and pooled serum was 2.81%, 3.17% and 10.30%, respectively. Within-laboratory precision was 4.33%, 4.24% and 8.86%, respectively. In linearity test, the coefficient of determination (R^2) was 0.9998, ranging from 14 to 54,301 mAU/mL. In comparison, the coefficient of comparison (r) was 0.9644 (between HISCL-5000 and LUMIPULSE G1200), 0.9633 (between HISCL-5000 and ARCHITECT i2000), and 0.9561 (between LUMIPULSE G1200 and ARCHITECT i2000), respectively. Agreements was 93.4%, 97.6% and 94.6%, and the kappa values was 0.855, 0.945 and 0.882 between HISCL-5000 and LUMIPULSE G1200, between HISCL-5000 and ARCHITECT i2000, and between LUMIPULSE G1200 and ARCHITECT i2000, respectively. The cut-off level of PIVKA-II was 40 1 mAU/mL and 98.4% of healthy individuals were below the cut-off value. **Conclusions:** PIVKA-II assay using HISCL-5000 showed acceptable analytical performance including precision, linearity and method comparison. This indicates that HISCL-5000 can be potentially helpful in clinical laboratories. 2

A-032

Low-end Precision Profile for a New PSAII Assay* on the ADVIA Centaur System and Comparison with Commercial PSA Assays *Under development. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

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Background: Total PSA assays are used to aid in the management (monitoring) of patients with prostate cancer. Among various treatments for prostate cancer is radical prostatectomy (RP). Guidelines define biochemical relapse after RP using a PSA threshold of 0.2 ng/mL (Adjuvant and Salvage Radiotherapy after Prostatectomy: ASTRO/AUA Guideline, 2013). Recent publications indicate that lower PSA thresholds after RP detect recurrence sooner (Kang, et al. J Urol. 2015, and Sokoll, et al. J Urol. 2016). The purpose of this study was to investigate low-end precision profiles of commercial PSA assays in comparison with a new ADVIA Centaur® PSAII Assay.* **Methods:** Sample pools were prepared by Siemens Healthineers targeted at 0, 0.005, 0.010, 0.015, 0.025, 0.050, and 0.200 ng/mL of total PSA. Pools were aliquoted and frozen at -70°C. Before testing, samples were thawed at 2–8°C and tested within 2 hours. Pools were run in replicates of five over 5 days on the Roche COBAS Modular Total PSA assay, Beckman ACCESS 2 HYBRITECH and WHO PSA assays, and Abbott ARCHITECT i1000 Total PSA assay at Hamilton Health Sciences Center. For ADVIA Centaur PSAII, pools were run in replicates of five over 5 days internally at Siemens Healthineers. **Results:** Precision was calculated in accordance with CLSI EP15-A3. Results are shown in the table below. Note that not all assays had the same mean for each pool, and differences in observed concentrations affected calculated CVs.

Pool	Abbott ARCHITECT i1000 Total PSA		Roche COBAS Modular Total PSA		ACCESS 2 WHO PSA[a]		ACCESS 2 HYBRITECH PSA[a]		ADVIA Centaur PSAII	
	Mean	Total % CV	Mean	Total % CV	Mean	Total % CV	Mean	Total % CV	Mean	Total % CV
1	0.001	63.9	0.002	110.3	0.00	NA	0.00	NA	0.000	NA
2	0.004	9.9	0.009	9.0	0.00	NA	0.00	184.5	0.003	31.2
3	0.008	7.5	0.013	7.0	0.01	0.0	0.01	0.0	0.007	17.8
4	0.019	5.3	0.029	3.4	0.02	0.0	0.02	0.0	0.023	5.3
5	0.039	3.6	0.055	3.3	0.02	9.0	0.02	11.6	0.048	4.1
6	0.147	3.0	0.197	1.4	0.17	2.8	0.18	2.8	0.191	2.4

NA: Not applicable. Results are below the lowest amount of signal the assay can detect. a. The ACCESS 2 assays report to two decimal places; thus precision at low concentrations cannot be calculated as accurately as for methods that report to three decimal places. **Conclusion:** The ADVIA Centaur PSAII assay demonstrated similar precision performance at the very low end of the concentration range compared to commercially available total PSA assays from Roche and Abbott.

A-033

Method comparison of CA 125 assay in two analyzers

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Background: Determination of serum CA125 is useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer. CA 125 assay values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and reagent specificity. We performed a method comparison study between two different analyzers, Unicell DxI 800 from Beckman Coulter® and Architect isr2000 from Abbott diagnostics®. The aim of this study is to evaluate the clinical concordance between both immunoassays. **Methods:** Measurements were performed in 101 serum samples from real patients. The samples were processed in parallel the same day at both analyzers. Statistical analysis was carried out with the MedCalc software, where the correlation was calculated by the Pearson´s coefficient, the Passing-bablok regression and Bland Altman plots. **Results:** Results from method comparison are resumed in the next data table

Test	Instrument	Study Unit	Component	N	Correlation coefficient Pearson r	Passing-Bablok Slope	Passing-Bablok Intercept	Deviation from linearity
CA-125	**x = CA125 DxI 800 Beckman Coulter y = CA125 isr2000 Abbott diagnostics**	U/mL	Serum	101	**0,9936** CI 95% = 0,9905 - 0,9957	**1,00** CI 95% = 0,96 - 1,04 **1 included**	**2,40** CI 95% = 1,74 - 3,47 **0 not included**	**P = 0,70 No significant deviation from linearity**

Results show a high degree of correlation coefficient and adjustment to linearity; however, there exists a constant bias. It would be necessary to check the clinical concordance of the results, checking if this bias could be ignored under our working standard conditions or we need to use a correction factor. Clinical concordance at the diagnostic according to cut-off (35 U/mL) is 99% (100/101). **Conclusion:** Method comparation results show a good correlation between both methods. Due to the high clinical concordance at the diagnostics, the bias we found in the method comparison could be ignored and the interchangeability of methods is possible. However, changes observed in serial CA 125 assay values when monitoring ovarian cancer patients should be evaluated in conjunction with other clinical methods used for monitoring ovarian cancer patients.

A-034

Lot-to-lot variability of the Binding Site Freelite® assays on the Optilite® and SPAPLUS® analysers

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Introduction: In the absence of a commutable international standard for serum free light chains (FLCs), Binding Site has produced a commutable bulk sample (gold standard) for the internal performance assessment of Freelite assays on multiple instruments. Its role is to ensure accurate and precise measurements that can be replicated across multiple instruments. Here we present the performance of the gold standard and inter-lot variation for 3 randomly selected Freelite assay lots released between June 2016 and October 2017 on the Binding Site Optilite and SPAPLUS analysers. Method: The performance of kappa and lambda Freelite assays on the SPAPLUS and Optilite analysers was assessed prospectively during routine batch manufacture by measuring 68 samples from healthy adult donors, 30 unprocessed and 27 processed panel samples (concentration range: 3-180 mg/L and 6-165 mg/L for κ and λ FLCs, respectively). A commutable gold standard reference material was also analysed. Three randomly selected assay lots released between June 2016 and October 2017 were included in the analysis. Results between lots

on the same platform were compared using Analyse-it® (Passing Bablok, linear regression and Altman Bland analyses) and sigma metrics calculated based upon total allowable error (Braga et al. Biochimica Clinica 2013;37:376-82). Results: Analyse-it results are shown in Table 1. Analytical process performance for three Kappa Freelite lots gave sigma values of 5.2, 5.9 and 9.1 on the Optilite and 7.3, 3.0 and 5.1 on the SPAPLUS. For three Lambda lots, sigma values of 7.0, 10.6 and 6.3 were obtained on the Optilite and 23.7, 7.2 and 18.4 on the SPAPLUS.

Table 1: Passing Bablok, linear fit and Altman-Bland results

Platform	Analysis	Kappa free		Lambda free	
		Lot 1 vs Lot 2	Lot 2 vs Lot 3	Lot 1 vs Lot 2	Lot 2 vs Lot 3
Optilite	n	106	105	118	115
	Passing Bablok slope and intercept	0.95 - 0.17	1.01 + 0.92	0.99 - 1.45	0.93 + 0.46
	Linear fit r value	0.996	0.997	0.996	0.998
	Altman Bland bias	-6.9%	7.6%	-9.1%	-4.4%
SPAPLUS	n	113	110	124	122
	Passing Bablok slope and intercept	1.02 + 1.37	1.02 - 1.43	1.01 - 1.11	1.02 + 0.18
	Linear fit r value	0.998	0.994	0.999	0.993
	Altman Bland bias	9.6%	-5.6%	-5.5%	2.2%

Conclusion: Inter-lot agreement for Optilite and SPAPLUS Freelite assays were within acceptable limits. With the exception of one SPAPLUS Kappa lot, all sigma values were >5. In future, the commutable gold standard internal reference material will be included in Freelite assay manufacture.

A-035

Serum free light chain analysis using the Optilite®analyser: a clinical laboratory perspective

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Background: Serum free light chain (sFLC) assessment has become routine laboratory practice, aiding in the clinical management of patients with monoclonal gammopathies. Monoclonal FLCs are not simple analytes to measure, with serum concentrations ranging from <10 mg/L to >10000 mg/L. Very high values may be attributed to FLC aggregation and, as with all immunoassays, high sFLC concentrations may result in underestimation due to antigen excess (AgXS). The structural diversity of monoclonal FLCs can also cause sample non-linearity. Here we determine the frequency of these issues and evaluate Freelite® assay performance on the Optilite in a routine laboratory setting. **Methods:** κ and λ sFLCs were measured in patient serum tested in our laboratory between 10/11/2016 and 22/12/2017 using Freelite assays on the Optilite (The Binding Site Group Ltd., UK). Overall there were 18418 κ sFLC measurements from 15353 samples and 18453 λ sFLC measurements from 15478 samples. We assessed the proportion of FLC values falling within the following ranges: ≤100 mg/L, >100-1000 mg/L, >1000-10000 mg/L, >10000 mg/L, and the number of samples requiring re-dilutions. The incidence of AgXS was determined by establishing the frequency of 'high activity' flagged samples and comparing the result to the value obtained at the next dilution; a higher result confirmed AgXS. Any observed cases of AgXS which were undetected by the analyser were noted. Non-linearity was evaluated for samples which gave a > value at a given dilution and a lower measurement at the next higher dilution; notable non-linearity was determined if the value was >30% lower. **Results:** Median κ and λ FLC concentrations were 27.94 mg/L (range 0.68-33649.00) and 19.25 mg/L (range 1.47-50638.00), respectively. Only 0.12% of κ and 0.08% of λ Freelite values were >10000 mg/L. Freelite values >30000 mg/L were extremely rare (5/15353 (0.03%) for κ and 13/15478 (0.08%) for λ). Overall, Freelite gave a final result in 83.12% (12761/15353) of κ and 82.63% (12790/15478) of λ samples at the standard dilution (1/10 measuring range (MR): 2.9 - 127 mg/L for κ; and 1/8 MR: 5.2 - 139 mg/L for λ). The next higher dilution provided final results for 13.33% of κ (1/100 MR: 29-1270 mg/L), and 8.70% of λ assays (1/80 MR: 52 - 1390 mg/L). Overall 267/36871 (0.72%) Freelite measurements (180/18418 [1%] κ and 87/18453 [0.5%] λ) were non-linear. Of these, the median percentage difference between the results at a given and next higher dilution was 10.57% (range 0.02%- 50.67%). Notable non-linear results were observed for only 0.06% of κ and 0.07% of λ Freelite measurements. In total, 11.16% of κ and 5.5% of λ measurements were flagged by the Optilite for re-dilution due to potential AgXS; which was confirmed in 94.7% of κ and 98.9% of λ cases. We only observed one case of undetected AgXS (1/30831; 0.003%) during the 1-year study period. **Conclusion:** Very high FLC values are rare and >80% of samples provide a final result for κ and λ Freelite values using standard Optilite dilutions. Notable non-linear FLC results are infrequent (<0.1%) and the Optilite provides robust Freelite AgXS detection.

A-036

Performance evaluation of cancer panels on the Alinity i system

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Background: Abbott Laboratories offers a wide variety of assays to test for many types of cancers including, breast, colon, gastrointestinal, liver, ovarian, pancreatic, testicular and prostate cancer. The Abbott oncology solution can help reduce laboratory operational costs while maintaining the quality standards necessary to have a meaningful positive impact to the quality and cost of health care. The Alinity ci system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 200 tests per hour The Alinity ci system has an increased reagent load capacity, holding up to 47 IA reagents, onboard QC, clot and bubble detection ability, and a dedicated pretreatment lane to provide consistent and reliable results. **Objective:** To demonstrate the analytical performance of representative assays of the Cancer Panel of the Alinity i system which consists of assays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for the quantitative determination of analytes in human serum or plasma. **Methods**: Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison were assessed per Clinical and Laboratory Standards Institute (CLSI) protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met. **Results:** The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the Cancer Panel are shown in the table below.

Assay	Total %CV	LOQ	Method Comparison to ARCH. (Slope/r)	Measuring Interval
Total Prostate Specific Antigen (PSA)	≤ 6.2	0.025 ng/ml	0.99/1.00	0.025 – 100.000 ng/ml
Free Prostate Specific Antigen (PSA)	≤ 7.9	0.021 ng/ml	1.06/1.00	0.021 – 30.000 ng/ml
CYFRA 21-1 (Cytokeratin 19)	≤ 5.2	0.15 ng/ml	0.95/1.00	0.50 – 100.00 ng/ml
PIVKA (Protein induced by Vitamin K absence)	≤ 3.2	7.60 mAU/mL	1.00/1.00	7.60 – 30000.00 mAU/mL
PROGRP (Pro-gastrin-releasing peptide)	≤ 4.7	0.93 pg/mL	0.99/1.00	0.93 – 5000.00 pg/mL

Conclusion: Representative immunoassays utilizing CMIA technology on the Alinity i system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT (ARCH) immunoassays.

A-037

Performance of CA72-4 Assay on Fully-automated Chemiluminescent Immunoassay Analyzer* (*In-development)

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Background: CA72-4 assay is widely used for monitoring of Gastric cancer and Ovarian cancer. CA72-4 antigen is a mucin-like, tumor-associated glycoprotein, also called as TAG72. The antigen is defined by 2 antibodies(CC49 and B72.3) which recognize its glycochain epitopes; Galβ(1-3) sialyl Tn and sialyl Tn antigens. The level in blood is elevated in some cancer patients(Ovarian cancer, Gastric cancer, Colon cancer etc.). **Objective:** To evaluate the quantitative analytical performance of newly developed CA72-4 assay (prototype ARCHITECT CA72-4 as-

say) on the fully-automated chemiluminescent immunoassay analyzer. **Material and Methods**: The measuring intervals of prototype ARCHITECT CA72-4 assay cover between 1.0 U/mL to 300 U/mL. Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison with on-market of Roche Cobas CA72-4 were assessed per CLSI protocols. **Results:** Total imprecision, LoQ, and linearity results are shown for ARCHITECT CA72-4 in the table below. Results versus on-market product demonstrated a slope 0.97 and r = 0.96.

Evaluation Item	Result
Total %CV	2 - 5 %CV
LoQ	0.6 U/mL
Linearity	1.0 – 300U/mL
Hook effect	Hook effect is not observed (to 20,000U/mL)
Cross reactivity	Cross reactivity is not observed. (AFP 2090ng/mL, bHCG 550mIU/mL, CA125 3.85KU/mL, CA15-3 836U/mL, CA19-9 4070U/mL, CEA 550ng/mL, CYFRA21-1 110ng/mL)
Interference	Interference is not observed. (Bilirubin, Hemoglobin, Protein, Triglyceride, Biotin)
Available tube type	Serum, Plasma (Serum plain, SST, EDTA-K3, EDTA-K2, EDTA-Na2, Li-Heparin and Na-Heparin)

Conclusion: The ARCHITECT CA72-4 assay demonstrated good precision, LoQ, Linearity, on-board stability, hook effect and Method comparison with on-market comparator assay.

A-038

Electrophoretic and nephelometric methods for monitoring monoclonal free light chains

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Background: Measuring monoclonal free light chains (FLCs) for monitoring response in multiple myeloma relies on urine protein electrophoresis (UPE) and urine immunofixation (uIFE) methods, and on serum FLC (sFLC) assessment using nephelometric/turbidimetric approaches. By contrast, serum protein electrophoresis (SPE) and immunofixation (sIFE) are of limited utility because sFLC levels are often below the sensitivity of these techniques. The highest concentrations of monoclonal FLCs in serum are typically found in light chain multiple myeloma (LCMM) patients. We have assessed the sensitivity of SPE/sIFE for identifying disease and for monitoring monoclonal serum FLC levels in a population of newly diagnosed LCMM patients enrolled onto the IFM-2009 trial, and compared the results to those by UPE/uIFE and by sFLC nephelometry. **Methods:** We included 101 patients with matched data for all five techniques (SPE, sIFE, UPE, uIFE and sFLC) at diagnosis. SPE, sIFE, UPE and uIFE were performed using standard laboratory procedures (Sebia, France). sFLC concentrations were measured nephelometrically using κ and λ sFLC Freelite® assays (The Binding Site Group Ltd, UK). sFLC measurements were considered abnormal if they were outside the manufacturer's reference ranges: κ sFLC (3.3-19.4 mg/L), λ sFLC (5.7-26.3 mg/L), κ/λ sFLC ratio (0.26-1.65). Minimal residual disease (MRD) was assessed by 7-colour flow cytometry after consolidation therapy (bortezomib, lenalidomide and dexamethasone). **Results:** M-protein was identified in 101(100%) screening samples either by an abnormal κ/λ sFLC ratio or positive uIFE, but only in 83(82%) patients by sIFE. UPE and SPE were positive in 79(78%) and 29(29%) patients, respectively. Median levels of involved FLC (3100 vs. 1525 mg/L; p=0.02), Bence Jones protein (1.95 vs. 0.73 g/24h; p=0.01) and total urine protein (2.89 vs. 0.51 g/24h; p<0.01) were significantly higher in SPE positive vs. negative patients, respectively. All patients had measurable sFLC levels (involved FLC >100 mg/L) at diagnosis, meeting current criteria for monitoring haematologic response, and 63% patients had measurable UPE (>200 mg/24h). By contrast, no patients had measurable disease (>10g/L) by SPE. In 77 patients with matched data at the end of consolidation, the κ/λ sFLC ratio remained abnormal in 41(53%) patients; and 15(20%) continued displaying elevated involved FLC levels. uIFE and UPE were positive in only 8(10%) and 1(1%) patients, respectively, whereas 7(9%) and 1(1%) had positive sIFE and SPE, respectively. The positive SPE and UPE result was from 2 different patients; both were lambda patients and had involved FLC >100 mg/L. In a subset analysis of 59 patients assessed by flow cytometry, 16(27%) were MRD-positive, consistent with the presence of malignant bone marrow plasma cells; 15/16(94%) had an abnormal κ/λ sFLC ratio and 7/16(44%) had elevated involved FLC. By contrast uIFE and UPE were positive in 3/16(18%) and 0/16(0%) patients, and sIFE and SPE in 2/16(12%) and 1/16(6%), respectively. **Conclusion:** FLC measurements using serum Freelite and urine immunofixation demonstrate good diagnostic sensitivity for identifying disease in LCMM; however sFLC assessment shows superior sensitivity for monitoring and better agreement with bone marrow assessment. Serum electrophoresis lacks sensitivity both for screening and monitoring and is therefore an unreliable method for monoclonal FLC assessment.

A-039

Heavy/Light Chain Assay In The Monitoring Of Multiple Myeloma.

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Introduction:
Serum protein (SPE) and immunofixation electrophoresis (IFE) have been extensively validated for the routine use of identifying, characterizing and quantifying monoclonal proteins. However, in particular, accurate quantitation of IgA monoclonal proteins can be difficult when they migrate in to the β fraction, due to co-migration with transferrin and complement components. The heavy/light chain immunoassay (HLC) is an additional tool for measuring intact immunoglobulin monoclonal proteins. Therefore, we aimed to examine the clinical utility of the HLC assay for the monitoring of IgG and IgA multiple myeloma (MM) patients.
Methods:
A total of 177 samples from 30 MM patients (21 IgG and 9 IgA) were analysed retrospectively with median number of 6 follow up samples per patient (range 3 – 13). Serum free light chains (sFLC) and heavy/light chains (HLC) were quantified using Freelite® and Hevylite® immunoassays (The Binding Site group Ltd, UK) run on the SPAPLUS turbidimeter (The Binding Site Group Ltd, UK). Details of M-protein concentration, beta globulin levels, total immunoglobulins levels and disease treatment response were obtained from the laboratory and patient information system. Passing-Bablok regression analysis was performed to compare (i) M-protein quantification with involved HLC (iHLC) and (ii) total immunoglobulin with summated HLC pairs for each immunoglobulin type (e.g. IgGκ+IgGλ). Statistical analysis was performed using MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).
Results:
For 127 IgG MM samples, IgG iHLC levels showed a good correlation with SPE quantification (iHLC y=0.96x+4.9; r=0.917) and summated HLC showed a good correlation with total IgG concentration (summated HLC y=0.94x+5.74; r=0.91). In total, 95/127 (75%) IgG MM follow up samples had an abnormal HLC ratio and 122/127 (96%) had a positive SPE, possibly due, in part, to the effects of IgG FcRN receptors recycling IgG and causing persistence of circulating IgG M-protein, even after tumour cell eradication. Consistent with this, one patient assigned a VGPR by IMWG criteria would be assigned a CR based on HLC measurements. For 50 IgA MM samples, 42/50 (84%) had an abnormal HLC ratio. Conversely, 50/50 (100%) of M-proteins showed β fraction migration and were difficult to accurately quantify by SPE. Therefore, M-protein concentration and iHLC did not correlate as well in IgA MM (y=1.9x-8.4; r=0.8) compared to IgG MM. However, there was good correlation between total IgA and summated IgA HLC (IgAκ+IgAλ y=1.35x-0.33; r=0.95). Of the 8/50 (16%) IgA samples with a normal HLC ratio, 6/8 (75%) were consistent with the disease status being in complete remission. Interestingly, in one IgA MM patient, SPE and IFE were negative but the serum FLC ratio and involved FLC were highly abnormal, possibly consistent with the presence of light chain escape.
Conclusion:
Our data suggests HLC measurements could add value to the current monitoring of multiple myeloma patients. In IgG MM patients, the M-protein level correlated well with HLC values. The HLC assay complements the serum FLC assay and is especially useful for monitoring of IgA MM patients who display M-proteins migrating in the β region on SPE.

A-040

Two novel technology approaches for the quantification of cancer miRNA biomarkers in clinical laboratories.

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Background:
MiRNAs have an immense potential to serve as diagnostic, prognostic and prediction biomarkers in the whole field of oncology. Their utilization as circulating biomarkers in non-invasive diagnostics is very promising as well. However, to our knowledge, no miRNA-based diagnostics is routinely used in clinical laboratories. We believe the reason is simple - currently used methods for miRNA determination and quantification are either low in specificity and sensitivity or they are very expensive and high technology demanding. Moreover, these methods are not easy to use, fast and reproducible. Thus, the need for new technologies is emerging.
Methods:
We are introducing new methods for absolute quantification of miRNA suitable for clinical use. The first method is named miREIA® and it is based on immunoassay format, which is very similar to well-known ELISA. It involves hybridization of target miRNA isolated from a patient sample to complementary biotinylated DNA oligonucleotide probe. The DNA/miRNA hybrids are then transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched DNA/miRNA hybrids. Next, the solid phase is incubated with streptavidin-HRP conjugate and the resulting complexes are visualized by a chromogenic substrate. Another approach in miRNA measurement utilizes the enzyme *Chlorella virus* DNA ligase (SplintR® ligase, NEB). This two-step method involves ligation of two adjacent DNA oligonucleotides hybridized to a miRNA target isolated from patient sample, followed by real-time quantitative PCR (RT-qPCR). The use of two PCR detection chemistries (more cost-effective SYBR green or more specific TaqMan probe) is possible.
Results:
Based on these two principles, we have developed assays for quantification of three onco-miRNAs (hsa-miR-23a-3p, hsa-miR-93-5p, hsa-miR-142-5p). Methods showed strong correlation with the TaqMan qPCR assay, where RNA isolated from whole blood and peripheral blood mononuclear cells was used. Both methods also displayed excellent analytical characteristics and high sensitivity. The calibration range of the miREIA® was 0.04-12.5 amol/µl with sensitivity lower than 0.13 amol/µl. The dynamic range of SplintR® ligase qRT-PCR was 7 logs and sensitivity 1 amol/µl.
Conclusion:
We conclude that the novel assays for miRNA quantification, miREIA® and SplintR® ligase qRT-PCR, meet elementary analytical and performance requirements for clinical laboratory methods and are potentially useful in clinical diagnostics.
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A-041

Total Antioxidant and Thiol Levels in Prostate Cancer Patients

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Background: Thiol groups are important anti-oxidants and essential molecules protecting organism against the harmful effects of reactive oxygen species (ROS). The aim of our study is to evaluate thiol-disulphide homeostasis with a novel recent automated method in patients with localized prostate cancer (PC) before and six months after radical prostatectomy (RP). **Material and Methods:** 18 patients with PC and 17 healthy control subjects were enrolled into the study. Blood samples were collected from the controls subjects and patients before and six months after RP. Thiol-disulphide homeostasis was determined using a recently developed novel method. Prostate-specific antigen (PSA), albumin, total protein, total thiol, native thiol, disulphide and total antioxidant status (TAS) were measured and compared between the groups. **Results:** Native thiol, total thiol and TAS levels were significantly higher in the control group than the patients before RP (p<.001). There was a non-significant increase in the native thiol, total thiol and TAS levels in the patients six months after RP in comparison to the levels before RP (p values 0.3, 0.3 and 0.09, respectively). We found a significant negative correlation between PSA and thiol levels. **Conclusion:** Our study demonstrated that the decreased thiol and TAS levels weakened anti-oxidant defence mechanism in the patients with PC as indicated. Increased oxidative stress in prostate cancer patients may cause metabolic disturbance and have a role in the aetiopathogenesis of prostate cancer.

A-042

A Novel Score Based on serum apolipoprotein A-1 & C-reactive protein is a prognostic biomarker in hepatocellular carcinoma patients

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Background: The aim of this study was to purpose a prognostic system based on preoperative serum apolipoprotein A-1 and C-reactive protein (ApoA-1 and CRP, AC score), and to evaluate the prognostic value in hepatocellular carcinoma(HCC) patients. **Methods:** Continuous 539 cases diagnosed with HCC from 2009 to 2012 in Sun Yat-sen University Cancer Center were analyzed. Characteristics, pretreatment lipids (ApoA-1, apolipoprotein B(Apo-B), high-density lipoprotein cholesterol(HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG)) and CRP levels were reviewed, and determined by univariate and multivariate Cox hazard models. Then the AC score was proposed, which combined the independent risk factors (ApoA-1 and CRP). **Results:** The optimal cut-off points in our study were evaluated by reference ranges. Patients with decreased ApoA-1 level(<1.090g/L), increased CRP level(≥3.00mg/L) had significantly poor overall survival (OS) and disease-free survival (DFS). The AC score was calculated as follows: patients with decreased ApoA-1 and elevated CRP were allocated as Score 3, patients with only one of these abnormalities were allocated as Score 2, and with no abnormalities were allocated as Score 1. Patients with higher AC score showed more progressed disease and poorer prognosis, not only in in the entire cohort (For OS, P<0.001; For DFS, P<0.001) but also in the subgroups stratified by pathological stage (stage I-II and stage III-IV). The discriminatory ability of AC score in HCC was assessed by AUC values, AC score (AUC: 0.676, 95%CI: 0.629-0.723, P<0.001) was higher than that of AFP. In addition, the combination of AFP and AC score (AUC: 0.700, 95%CI: 0.655-0.745, P<0.001) was superior to that of AFP, AC score only. **Conclusions:** The AC score is significantly valuable predictor of OS and DFS, and could more accurately differentiate the prognosis of HCC patients. As this study is a retrospective analysis, and the value of AC score should be validated in large prospective trials.

A-043

Heat shock proteins 90α provides a novel and effective diagnosis therapeutic strategy for Nasopharyngeal carcinoma

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Background: EBV infection is closely related to the occurrence of Nasopharyngeal carcinoma (NPC). Previous studies in our research group found that the positive predictive value of EBV antibody for NPC is not ideal. Heat shock proteins (HSP) are a family of proteins which have been produced by many cells, including tumor cells, which is associated with tumorigenesis and tumor progression. There is an urgent need for non-invasive, high-performance biomarkers in clinical laboratory medicine to aid the diagnosis of NPC. In this study, this is the first time to investigate the role of heat shock protein Hsp90α in the diagnosis and progress of nasopharyngeal carcinoma (NPC). **Methods:** Hsp90α was detected in 196 newly diagnosed NPC patients, 76 corresponding post-treatment NPC patients, 230 VCA-IgA positive normal subjects and 106 healthy donors by ELISA. NPC patients group: Between September 2016 and December 2016, 196 untreated NPC patients pathologically diagnosed at Sun Yat-sen University Cancer Center.Clinical stage (2008 staging system): 8 patients in stage I, 16 patients in stage II, 97 patients in stage III and 75 patients in stage IV. EBV VCA IgA were positive in the NPC patients; All the people in our study have similar age, gender, place of residence, place of origin, and smoking history) **Results:** (1) The level of Hsp90α in plasma of 196 patients with NPC was (212.16 ± 144.32) pg/ml, which was significantly higher than VCA-IgA positive normal subjects(68.12±64.94 pg/ml,P<0.001)and healthy donors(35.87±17.47 pg/ml, P<0.001. The level of Hsp90α in plasma of VCA-IgA positive normal subjects was significantly higher than that in healthy donors (P <0.001). (2) The level of Hsp90α in plasma of patients with NPC in the early stage (I+II), stage III and stage IV was 159.69 ± 117.12

pg / ml, 195.24 ± 126.38 pg / ml and 250.85 ± 164.66 pg / ml, respectively. The level of Hsp90α in plasma of patients with NPC in early stage (I + II) and stage IV, stage III and stage IV were significantly different (P = 0.018, P = 0.029); The level of Hsp90α in plasma in patients with metastasis of NPC and those without metastasis was significantly different (P<0.001). (3) The level of Hsp90α in plasma of patients with NPC before and after treatment were significantly different (212.16 ± 144.32 pg / ml vs. 62.36 ± 34.04 pg / ml, P <0.001). (4) The ROC curves demonstrated that the sensitivity of plasma Hsp90α in distinguishing NPC patients from healthy donors was 74.50% and the specificity was 99.10%(AUC=0.931, 95%CI:0.903-0.958); The sensitivity of plasma Hsp90α in distinguishing NPC patients from VCA-IgA positive normal subjects was 74.50% and the specificity was 81.70% (AUC = 0.831, 95% CI: 0.790-0.871). **Conclusions:** Hsp90α is closely related to the clinical stage, metastasis and therapeutic effect of NPC, so it may serve as a new biomarker for diagnosis and treatment of NPC .

A-044

Identification of oncogenic driver mutations in non-small-cell lung cancer patients

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Introduction: Lung cancer is the leading cause of cancer-related deaths worldwide. Identification of genetic aberrations critical to cancer development and maintenance (oncogenic driver mutations) has transformed care in lung cancer patients. Next generation sequencing (NGS) has served as a powerful tool in identifying genetic mutations. Targeted therapies including small molecule inhibitors and antibodies against these driver mutations are currently serving as personalized therapy and are preferable to standard chemotherapy.**Objective**: We determined the frequency of oncogenic drivers in patients with non-small cell lung cancer in an endemic region and their association with age, sex and actionable therapy **Method**: We retrospectively analyzed patients who underwent targeted NGS using a targeted NGS platform (FoundationOne CDx™) between October 2013 and December 2017. Percentage association between mutation status, age, sex and actionable therapy was performed. **Results**: Of the 88 lung cancer patients tested for driver mutations, 2 (2%) patients had no reported genetic alterations. Patients included lung adenocarcinoma (ADC), 66 (75%); squamous cell carcinoma (SCC), 16 (18%); large cell carcinoma (LCC), 3 (3%); atypical carcinoid (ATC), 1 (1%) and sarcomatoid carcinoma (SAC), 1 (1%). Among 85 patients studied, top driver mutations included *TP53*, 55 (65%); *KRAS*, 26 (31%); *STK11*, 16 (19%), *CDKN2A*,14 (16%); *LRP1B*, 10 (12%); *PIK3CA*, 12 (10%); *NF1*; 9 (11%) and *EGFR*, 8 (9%). Sixteen (19%; 14% ADC, 4% SCC and 1% LCC) patients were found with potentially actionable genetic alterations. Of the 496 total mutations, 256 (52%) were in females and 241 (49%) were in males. Mean number of mutations per patients was 5.6 (95% CI, 4.7-6.5) including ADC 4.9 (95% CI, 4.0-5.9) and SCC, 8.4 (95% CI, 6.2-10.7). **Conclusion**: The genomic landscape of our patient's tumors is consistent with previously reported studies and, importantly, emphasizes that 19% (14% ADC, 4% SCC, 1% LCC) of patients had actionable driver mutation. This suggests that identification of these mutations can identify patients who will benefit from targeted therapy, emphasizing the role of laboratory medicine in enabling personalized onco-therapeutics. Additional driver mutations with potential targeted therapies were also identified, however, randomized trials are required for further evaluation.

A-045

Novel Tissue-specific Autoantigens Associated with Clinical Outcomes in Response to PD-1/L1 Directed Immunotherapy in NSCLC.

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Objective: The impressive clinical outcomes resulting from PD-1/-L1 directed immune-checkpoint inhibition has led to a recent paradigm shift in the treatment of advanced non-small cell lung cancer (NSCLC). While tumoral PD-L1 expression remains the only prognostic biomarker to select patients for this therapeutic strategy, the limited performance of this marker spurns on the search for improved molecular diagnostics. The primary objective of this study is to identify candidate biomarkers to better prognosticate response to PD-1/-L1 directed immunotherapy based on differences in a humoral response to the tumor. Resulting autoantibodies **Method:** Lysates from A549 lung adenocarcinoma cells were resolved via 2-dimensional IEF/SDS-PAGE electrophoresis to address our primary endpoint, with 3 gels total performed in parallel: 1 stained for protein and 2 transferred to nitrocellulose. Individual membranes were immunoprobed with pooled pretreatment sera (n=4/group) derived from patients with advanced NSCLC receiving PD-1/-L1 directed immunotherapy that either have documented disease progression within 6 weeks of induction or have radiographically stable disease or better in the first 12 weeks of therapy. Immunoreactive spots were detected with a HRP-conjugated, anti-human IgG secondary antibody and developed via ECL reagents. Differentially immunoreactive spots were evaluated via densitometry in PDQuest (BioRad) with a 4-fold difference in expression threshold used to prioritize spots for identification via LCMS at the Mass Spectrometry Facility at the University of Illinois, at Chicago. As a secondary objective, we also performed this same maneuver contrasting autoantibodies in isolated from pretreatment and 12-week post induction time points, from each group. **Results:** We identified series of differentially expressed autoantigens that are candidate biomarkers for prognosticating clinical outcomes for advanced NSCLC patients receiving PD-1/-L1 directed immunotherapy. Our primary endpoint in this study was to identify circulating autoantibody biomarkers in pretreatment sera that have value for prognosticating a "good" versus "poor" clinical outcome. For this, we identified five autoantibodies in pretreatment sera using immunoproteomic methods that were associated uniquely with stable disease in the first 12 weeks of PD-1/-L1 directed immunotherapy. The corresponding autoantigens were identified via LCMS as heat shock protein A4, transitional endoplasmic reticulum ATPase, mitochondrial NADH-ubiquinone oxidoreductase 75 kDa subunit, heat shock protein D, and glyoxalase domain containing protein 4. In parallel, we also identified antibodies against annexin A1 as being uniquely associated with progressive disease. For our secondary objective, we were able to identify a series of circulating autoantigens capable of monitoring treatment response over the first 12 weeks of therapy. Specifically, we found retinaldehyde dehydrogenase 1, stress-induced-phosphoprotein 1, and annexin A2 as being highly correlated with disease progression in patients receiving PD-1/-L1 directed immunotherapy. Only autoantibodies against UDP-glucose 6-dehydrogenase were identified as a candidate biomarker for monitoring patients receiving clinical benefit from immunotherapy over the first 12 weeks of treatment. **Conclusion:** A series of candidate (autoantibody) biomarkers with value for prognosticating patient response to PD-1/L1 directed checkpoint inhibition. These targets are currently being developed into multiplexed immunobead assays for evaluation across appropriate cohort of advanced NSCLC patients receiving immunotherapy that are archived in our institutional biorepository.

A-046

Clinical Correlation between Serum Biomarkers CA27.29 and CA15-3 and Disease Status in Patients with a History of Advanced Breast Cancer

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Background: MUC1 is a biomarker used to aid in detection of recurrence and monitoring treatment response in patients with advanced breast cancer. MUC1 can be measured by two immunoassays: Cancer Antigen 27.29 (CA27.29) and Cancer Antigen 15-3 (CA15-3). While both immunoassays measure the same protein, they are sometimes ordered concurrently for patient management. Changes in MUC1 concentration between sequential measurements are monitored, with a significant percent change (%change) defined as 20–30%. Objective: The purpose of this study was to correlate concurrent CA27.29 and CA15-3 concentrations with clinical disease status to determine if one assay was superior in disease monitoring. Methods: In this IRB-approved study, 314 results were reviewed from 178 Mayo Clinic patients with a history of breast cancer. In each case, CA27.29 (ADVIA Centaur, Siemens Healthineers, Malvern, PA) and CA15-3 (Roche Cobas, Roche Diagnostics, Indianapolis, IN) were ordered on the same day during 2014–2015. Chart review was completed for 25 patients with multiple paired tumor marker (TM) orders and corresponding imaging results (n=63). Concordance between CA27.29 and CA15-3 results was evaluated. Positive results were defined as TM concentrations greater than the reference interval (RI, CA15-3: <30 U/mL, CA27.29: </=38 U/mL). Imaging studies corresponding to the time of TM sample collection were used to define disease status for the first measurement (baseline) and subsequent measurements. Baseline disease was classified as disease present (stable or progressing) or disease absent (no detectable disease). Subsequent statuses were defined as progressing (increase in disease), responding (decrease in disease), or stable (no change in disease). TM %change of 25% was defined as significant and used to correlate with disease status. TM %change was considered concordant with disease status change if: increase >/=25% for progressing, decrease >/=25% for responding, and <25% for stable. Results: The positive concordance between CA27.29 and CA15-3 was 100% (44/44), while the negative concordance was 90% (17/19). Twenty-one patients were classified as disease present and four as disease absent at baseline. CA15-3 and CA27.29 concentrations at baseline correlated with the disease status for nineteen (90%) of the disease present patients (CA27.29/CA15-3 > RI) and 100% of the disease absent patients (CA27.29/CA15-3 < RI). In 71% (27/38) of cases the subsequent measurement showed a %change in both TM's

that correlated with disease status. In the eight instances where neither TM correlated with disease status, six cases showed a decreased TM in the setting of disease progression and two cases had stable disease with a 25% or greater %change in TM at the subsequent measurement. There were six instances (16%) where only one TM correlated with disease status (three cases for each TM). Four of these cases were classified as stable disease. Conclusions: CA27.29 and CA15-3 results have strong concordance and overall similar clinical correlations. In cases where only one of the TM's correlated with the disease status of the patient, each TM correlated 50% of the time. The results from this patient cohort suggest that there is no clinical benefit to ordering both TM's concurrently for breast cancer patient management.

A-047

Serum HE4 levels in pancreatic and gastric cancer.

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Background: Pancreatic and gastric cancers are the seventh and the ninth most common type of cancer in Spain. CEA and Ca19.9 have been the worldwide tumor markers used in clinical practice. Human epididymal protein 4 (HE4) is a marker of ovarian and endometrial cancer, though studies at the tissue level have found an association between this marker and certain neoplasms of the digestive system such as gastric and pancreatic cancer. A little is known about serum levels of HE4 in this kind of pathologies. **Methods:** A total of 112 healthy individuals (47 men, 65 women) recruited as a control group, 49 patients diagnosed with pancreatic cancer (16 men, 33 women) and 33 patients diagnosed with gastric cancer (16 men, 17 women) were selected. None of the subjects presented kidney failure, gynecologic pathology, or other tumor types. The group of cancer was undergoing chemotherapy and/or surgical treatment at the time of the study. Serum determination of HE4, Ca19.9, and carcinoembryonic antigen (CEA) markers was performed in both groups. We compared median values and constructed receiver operating characteristic (ROC) curves, calculating area under the curve (AUC), sensitivity, specificity, and cut-off points for both groups. **Results:** Significant differences in HE4 ($p<0.001$) adjusted by age were observed in the group of pancreatic cancer patients, revealing an AUC of 0.92, a sensitivity of 87.8%, and a specificity of 82.3% with a cut-off point of 63.23 pmol/L. CEA and Ca19.9 showed significant differences ($p=0.001$ and $p=0.028$ respectively) adjusted by age revealing an AUC of 0.85 and 0.77 respectively.Significant differences in HE4 ($p<0.001$) was also found adjusted by age in the group of gastric cancer patients. AUC of 0.9, a sensitivity of 84.8% and specificity of 81.4% with a cut-off point of 62.73 pmol/L. Ca19.9 and CEA showed no differences between case and control group. **Conclusion:** In patients with pancreatic and gastric cancer, HE4 levels are higher when compared to the widely used markers Ca19.9 and CEA, although more studies are needed to clarify this association.

A-048

A Four Kallikrein Panel Test Accurately Predicts Risk of High Grade (Gleason score ≥7) in men with PSA 1.0 - 10.0 ng/mL

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Introduction

Prostate cancer is the most common cancer in men and is projected to account for 164,690 new cases and 29,430 deaths in the U.S. in 2018. Screening for prostate cancer with Total PSA (PSA) has been a significant contributor in reducing the overall mortality from prostate cancer by over 50% in the U.S., but questions remain as to the risk vs. benefit of PSA screening due to complications caused by over diagnosis and over treatment of non-lethal cancer. The complications associated with PSA screening begin with the prostate biopsy, which must be performed to diagnose prostate cancer. About 75% of all prostate biopsies find either no cancer or find Gleason score 6 cancer. A biomarker test is needed to effectively identify men likely to harbor aggressive prostate cancer. Such a test would improve the efficacy of PSA screening by reducing the use of prostate biopsy and the diagnosis of indolent cancer, while still showing high detection rates for aggressive cancers.

Materials and Methods

A new blood test (4Kscore) consisting of a panel of four kallikrein biomarkers (Total PSA, Free PSA, Intact PSA, and hK2), combined with clinical information in an algorithm, has been tested in two prospective U.S. clinical studies involving over 1,300 patients.[1,2] We report here for the first time a combined analysis of 1124 patients, representing a contemporary, racially diverse (19% African American) subgroup from these studies. All the men have a PSA 1.0-10.0 ng/ml. Based on their PSA and clinical information, the cohort represents a group identified in the "grey zone" by screening where the decision for prostate biopsy would benefit from further information of the patient's risk.

Results

The 4Kscore shows significantly better AUC performance in the detection of high grade disease (Gleason score ≥7) vs. use of PSA alone (0.776 vs. 0.638). At a 4Kscore cut point of 7.5%, the sensitivity and negative predictive (NPV) value were 92% and 95%, respectively. The PSA sensitivity and NPV (cut point 4.0 ng/mL) were 85% and 89%, respectively. The 4Kscore would also have spared 379 (34%) of the prostate biopsies. It is noteworthy that 4Kscore detected all Gleason score ≥8, while PSA at 4.0 ng/mL missed 7 out of 64 (11%).

Conclusions

Our study looked at the performance of the 4Kscore test compared to PSA alone to distinguish men with high grade prostate cancer. The cohort was a racially diverse cohort derived from two prospective U.S. clinical studies, and represents an important at risk population in need of further risk stratification. The 4Kscore showed significantly better AUC, sensitivity and NPV performance for predicting high grade prostate cancer at biopsy when compared with PSA.

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[2] Punnen S, et al. J Urology (2018), doi: 10.1016/j.juro.2017.11.113.

A-049

Monitoring EGFR mutations in cfDNA during different treatment lines in Non-small-cell lung-cancer (NSCLC) patients

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Background: The cobas® EGFR Mutation Test v2 is the only FDA approved test to qualitative detection in plasma from non-small cell lung cancer (NSCLC) patients for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR), which can benefit of the treatment with tyrosine kinase inhibitors (TKIs). However, patients under this therapy can eventually develop resistance, mainly due to the presence of T790M mutation. Since repeated biopsies are often an invasive procedure, analysis of EGFR mutations in blood becomes a useful tool to investigate mutational progress. **Objectives:** To assess the ability of cobas® EGFR Mutation Test v2 to detect EGFR mutations, concordance of matched-tissue and plasma and capability of use as a semiquantitative platform for treatment monitoring. **Methods:** Plasma samples from 21 EGFR positive NSCLC patients (13 males, 58 ± 12 years, 8 females, 63 ±11 years) were collected under informed consent. Blood was collected at baseline treatment baseline and at sequential timepoints, including progression and subsequent second line treatments. cfDNA isolation was performed with the cobas DNA Sample Preparation kit (Roche Molecular Systems, Inc. CA, USA). Concentrations of isolated cfDNA were measured in a Qubit 2.0 Fluorometer (Life Technologies) and characterized in a 2200 TapeStation system (Agilent Technologies, CA, USA). Mutations were tested with the cobas® EGFR Mutation Test v2. Mutations were also quantified by digital droplet PCR (ddPCR) (Bio-Rad). **Results:** Mean cfDNA was 0,28 ±0,19 ng/µL. Purity of cfDNA isolated was confirmed by a High Sensitivity D1000 ScreenTape® assay (175,2 ± 8,78 bp) as well as the absence of genomic DNA. We were able to detect EGFR mutations in ctDNA in 12/21 of tissue and blood paired samples. Regarding to plasma positive results, a complete overall concordance was found with tissue (12/12). We could also detect T790M resistance mutation in two patients at the baseline and, very interestingly, one of them could not be detected in its tumor-matched material. When analyzing progression samples, we also observed a gradual increase in Semi-quantitative index (SQI), although changes were not significant at progression. Also, in 3/21 negative cfDNA baseline patients, results turned positive in subsequent progression samples. We analysed results obtained by cobas technology and ddPCR in order to know if a correlation did exist between SQI and number of copies/mL respectively, but no correlation was found in neither of the mutations analyzed: del19, L858R, and T790M. **Conclusion:** cobas® EGFR Mutation Test v2 sensitivity is low but if positive, can avoid tissue biopsy. It can identify the existence of resistance mutations in patients without detectable mutation in biopsy. Sequential analysis at progression should also be taken in account as it can detect the presence of resistance mutation avoiding a rebiopsy.

A-050

Circulating tumor DNA detection with a novel platform for single molecule sequencing validated for targeted and immunotherapy selection

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Background
Comprehensive genomic profiling of solid tumors using circulating tumor DNA (ctDNA) has enabled the detection of all NCCN guideline-recommended somatic genomic classes of alterations from a single, non-invasive blood draw. However, current ctDNA tests still face two major challenges: the inability to reliably identify somatic variants at low mutant allele fraction (MAF), and inconsistency in how the tests have been validated. This study shows how the Single Molecule Sequencing (SMSEQ) platform addresses these challenges. The SMSEQ platform integrates innovative ctDNA extraction methodology, highly optimized library preparation and an error-based variant-calling algorithm to drastically improve sensitivity and specificity. The platform analyzes 5 classes of somatic variants: single nucleotide variants (SNVs), insertions and deletions (Indels), copy number variants (CNVs), fusions and microsatellite instability (MSI).
Methods
We analyzed a 73 gene panel covering NCCN recommended actionable variants for solid tumors in 60 reference ctDNA samples with known variants to establish the limit of detection, sensitivity, specificity, accuracy and reproducibility of the SMSEQ platform. For clinical validation, we tested 36 patients with metastatic colorectal cancer (mCRC) and 34 healthy controls from the Chang Gung Memorial Hospital, and 227 patients diagnosed with solid tumors from Taiwan. Circulating tumor DNA was extracted from plasma followed by library preparation using a highly optimized NGS workflow. Somatic variants in ctDNA are identified using locus-specific modeling to separate tumor variants from normal errors.
Results
Validation according to recently published ACMG/AMP guidelines, shows that the SMSEQ platform allows calling of variants with >99.999% analytical specificity for SNVs, Indels and fusions; and >99% analytical specificity for CNVs and MSI. The platform successfully detected variants at low MAF: 0.1% for SNVs and Indels, <1% for fusions, 5 copies for CNVs, and 1% for MSI.
Somatic variants were identified in 35 of 36 mCRC patient samples (97.2%). No false positives were observed within the targeted region for all 34 healthy controls tested. In paired samples, the SMSEQ platform showed 89.7% concordance with tissue biopsy. Observed gene mutation profiles from ctDNA were consistent with published tissue biopsy data: the most frequently mutated genes were *TP53, APC,* and *KRAS*; KRAS and BRAF variants were mutually exclusive. In addition to mCRC patients, we tested 227 patients diagnosed with various solid tumors from Taiwan. Actionable variants were detected in 170/227 (74.8%) patients.
Conclusion
The CellMax 73-gene liquid biopsy test, using the SMSEQ platform, detects 5 NCCN-guideline recommended variant classes: MSI for immunotherapy as well as SNVs, Indels, CNVs, and fusions for targeted therapy selection at low variant allele fraction/copy number at high sensitivity and specificity.

A-051

Clinical evaluation of HE4 in lung cancer diagnosis

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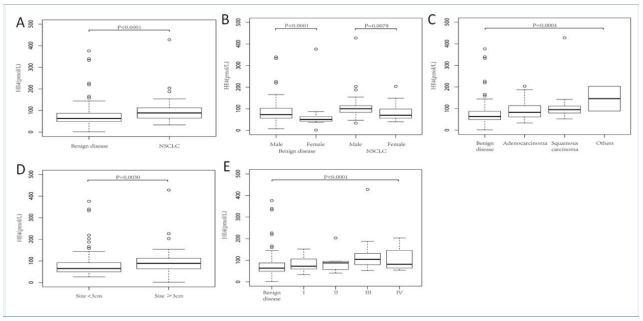
Background and Aims
Lung cancer is the most common cancer and the leading cause of cancer-related death worldwide. With the progress of tumor stage, the prognosis was significantly worse. Thus, early diagnosis of lung cancer is of importance to improve the outcomes of the patients. Human epididymis 4 (HE-4) has been recently shown to be a potential new biomarker for lung cancer. Thus, the present study is to investigate its clinical utility in lung cancer diagnosis.
Methods
A total of 67 non-small cell lung cancer(NSCLC) patients and 112 subjects with benign lung tumors , were recruited from the First Affiliated Hospital of Xiamen University. All the patients' information were recorded and validated. The study received ethical approval from the site. Serum HE4 was examined by the ARCHITECT i2000(Abbott,USA).
Results
Serum levels(Mean±SD) of HE4 in benign disease and NSCLC were 78.88±58.04 pmol/L and 135.89±230.19 pmol/L ($p<0.001$) respectively. Higer serum levels of HE4 were found in NSCLC compared to benign disease($p<0.001$, Fig.1A). Interstingly, HE4 levels in male were siginificantly higher than female in both group, suguesting the gender specific cutoff may be needed when using HE4 in disagnosis(Fig.1B). According to pathological types, HE4 levels in squamous carcinoma were higher than adenocarcinoma and benign disease(Fig.1C). Higher HE4 levels were also detected in larger tumor size (Fig.1D) and late stage(Fig.1E). ROC analysis showed an AUC of 0.675 with sensitivity of 0.612 and specificity of 0.688 at the cutoff of 79.5 pmol/L.
Conclusions
The preliminary data of this study supported the potential clinical application of HE4 in lung cancer diagnosis.



A-052

Multicenter Clinical Evaluation of New Free Light Chain Methods

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Background: The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free-light-chain (FLC) determinations for diagnosis and management of clonal plasma-cell disorders. We describe reproducibility, linearity, reference range determination, clinical sensitivity, and specificity data for two new immunoassays that detect FLC-type kappa and lambda, and their ratio.
Methods: Latex-enhanced mouse monoclonal antibody reagents from Siemens Healthcare Diagnostics Inc. for kappa (N Latex FLC kappa) and lambda (N Latex FLC lambda) were assayed on multiple BN™ II and BN ProSpec® Systems. Precision studies were conducted according to CLSI guideline EP5A-2 to estimate repeatability of three sample pools and two controls each for kappa and lambda, using up to three reagent and calibrator lots at four sites. A reference range study on U.S. populations included 201 serum samples from apparently healthy adults (47% female; 53% male; age 21-86 years). A validation reference interval study including 178 donors (59% female; 41% male; age 21-66 years) was conducted to ensure the first study's robustness. Clinical sensitivity of the N Latex assays was investigated against the clinical condition of patients at various disease stages in defined populations consisting of 96 multiple myeloma (MM) and 83 amyloidosis (AL) patients. For comparison, the same populations were investigated using the available FREELITE methods from The Binding Site for the BN II System. The specificity panel applied to the N Latex FLC methods consisted of 163 samples from patients with various immunological conditions. Method agreement for the new FLC and FREELITE BN II assays was evaluated using Passing-Bablok regression analysis.
Results: Between-lot/between-instrument reproducibility for the new kappa assay on the BN II System ranged from 3.5-6.0%/1.2-3.5%. On the BN ProSpec System, the kappa assay's between-lot/between-instrument results were 4.6-7.2%/4.0-7.0%. Between-lot/between-instrument lambda results on the BN II System were 5.9-9.2%/4.1-6.5%. On the BN ProSpec System, between-lot/between-instrument results for lambda were 2.6-7.1%/0.4-3.8%. Reference range studies showed kappa (κ) concentrations (2.5th percentile/median/97.5th percentile) of 8.24/15.1/28.9 mg/L and lambda (λ) concentrations of 9.1/17.3/32.6 mg/L; κ/λ ratio results were 0.53-1.51 (1st-99th percentile). Reference interval validation revealed within-range recoveries of 91.0% for kappa, 93.3% for lambda, and 96.6% for the κ/λ ratio, thereby confirm-

ing the validity of the initial ranges according to CLSI guideline C28-A3. Clinical sensitivity based on the κ/λ ratios for the N Latex FLC methods was 95.8% in the MM and 83.1% in the AL population. The respective results for the comparison methods were 95.8% and 77.1%. Clinical specificity was 96.9% for both N Latex methods. Passing-Bablok regression results between methods were y = 0.794x + 2.1 mg/L (r = 0.943) for kappa (n = 216) and y = 1.17x + 2.16 mg/L (r = 0.975) for lambda (n = 218). **Conclusion:** The new N Latex FLC methods showed acceptable and consistent precision over several reagent lots and instruments, valid reference ranges, and acceptable correlation with an FDA-cleared method. These methods are an attractive alternative for FLC measurement in clinical laboratories. HOOD05162002786441

A-053

Reliability of the SPAPLUS® Analyser for the Assessment of Serum Free Light Chains: PathCare Laboratory, South Africa

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Background: Serum free light chain (sFLC) assays (Freelite®) are included in both international and national guidelines for the diagnosis, monitoring and prognosis of multiple myeloma and related disorders. However, historically, sFLC assays run on a wide plethora of analysers exhibited well published antigen excess, non-linearity and over-estimation issues. The manufacturer of the assay has reportedly overcome some of these issues on optimised analysers, including the SPAPLUS. The aim of this study was to evaluate the performance of the Freelite assay on the SPAPLUS, in a large reference laboratory in South Africa, and report on the incidence of antigen excess, sample non-linearity and extremely high sFLC values. **Methods:** sFLCs were measured using κ and λ Freelite immunoassays (The Binding Site Group Ltd., Birmingham UK) on a SPAPLUS analyser (The Binding Site Group Ltd., UK) at PathCare laboratory, Cape Town, South Africa. All results obtained between 01/06/2017 and 18/09/2017 were included. The incidence of antigen excess was calculated based on the proportion of samples flagged with abnormal reaction kinetics by the automatic antigen excess check on the analyser. Extreme sFLC concentrations were defined as κ or λ sFLC concentrations >30,000 mg/L (cut-off based on the subtraction of a typical serum albumin concentration [40 g/L] from a typical serum total protein concentration [70 g/L]). Non-linearity was investigated when samples gave a > value at a 1/10 sample dilution, but a result within the 1/10 measuring range when assayed at a 1/100 dilution. Notable non-linear samples were defined as those that gave results that were >30% different at the two dilutions. **Results:** A total of 1452 serum samples from 1150 patients were included in the analysis. An abnormal κ/λ sFLC ratio (<0.26 or >1.65) was reported in 855/1452 (59%) of samples and indicated monoclonal κ sFLCs or monoclonal λ sFLCs in 87% and 13% of cases, respectively. Of the samples with monoclonal κ sFLCs, the mean κ sFLC concentration was 502 mg/L (range 4.38 mg/L- 34,300 mg/L). Of the samples with monoclonal λ sFLCs, the mean λ sFLC concentration was 1,414 mg/L (range 4.8 mg/L - 26,100 mg/L). In 83% of cases, samples gave a result at the initial online dilution. Antigen excess was flagged in 16/1452 samples (1.1%). Only 1/1452 samples (0.07%) had a sFLC concentration >30,000 mg/L. 72/1452 (5.0%) of samples were non-linear. Of these 72 samples, the median percentage difference between the result at 1/10 and 1/100 dilution was 15% (range 0.41% - 31.4%). Only 2/1452 samples (0.14%) had notable non-linearity. **Conclusion:** We conclude that analytical issues associated with monoclonal sFLC measurement are an infrequent occurrence in routine laboratory practice, when using the Freelite assays on the SPAPLUS analyser. We experienced very little sample non-linearity (0.14%), the incidence of antigen excess was low (1.1%) and very few samples had extremely high sFLC values (0.07%).

A-054

Establishment and validation of a predictive nomogram model for non-small cell lung cancer patients with chronic hepatitis B viral infection

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Background: This study aimed to establish an effective predictive nomogram for non-small cell lung cancer (NSCLC) patients with chronic hepatitis B viral (HBV) infection. **Methods:** The nomogram was based on a retrospective study of 230 NSCLC patients with chronic HBV infection. The predictive accuracy and discriminative ability of the nomogram were determined by a concordance index (C-index), calibration plot and decision curve analyses and were compared with the current TNM staging system. **Results:** Independent factors derived from Kaplan-Meier analysis of the primary cohort to predict overall survival (OS) were all assembled into a Cox's proportional hazards regression model to build the nomogram model. The final model included age, tumor size, TNM stage, treatment, apolipoprotein A-I, apolipoprotein B, glutamyl transpeptidase and lactate dehydrogenase. The calibration curve for the probability of OS showed that the nomogram-based predictions were in good agreement with actual observations. The C-index of the model for predicting OS had a superior discrimination power compared with the TNM staging system [0.780 (95% CI: 0.733-0.827) vs 0.693 (95% CI: 0.640-0.746), $P < 0.01$], and the decision curve analyses showed that the nomogram model had a higher overall net benefit than the TNM stage. Based on the total prognostic scores (TPS) of the nomogram, we further subdivided the study cohort into 3 groups: low risk (TPS ≤ 13.5), intermediate risk (13.5 < TPS ≤ 20.0) and high risk (TPS > 20.0). **Conclusion:** The proposed nomogram model resulted in more accurate prognostic prediction for NSCLC patients with chronic HBV infection.

A-055

Prognostic nomogram for patients with Nasopharyngeal Carcinoma incorporating hematological biomarkers and clinical characteristics

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Background: Predictive models for survival prediction in individual cancer patients following the TNM staging system are limited. The survival rates of the patients who have the same TNM stage disease were diversified. Therefore, we construct a nomogram that incorporates hematological biomarkers and clinical characteristics for predicting the overall survival (OS) of nasopharyngeal carcinoma (NPC) patients. **Methods:** The clinicopathological and follow-up data of 460 NPC patients who were diagnosed histologically in Sun Yat-sen University Cancer Center between July 2007 and December 2011 were retrospectively reviewed. The data was randomly divided into primary and the validation groups. Cox regression analysis was used to identify the prognostic factors for building the nomogram in the primary cohorts. The predictive accuracy and discriminative ability of the nomogram were measured by concordance index (C-index) and decision curve and were compared with the TNM staging system, Epstein-Barr virus DNA copy numbers (EBV DNA) and TMN stage plus EBV DNA. **Results:** The results were validated internally by assessing discrimination and calibration using the validation cohorts (N = 230) at the same institution. Independent factors for overall survival including Age [hazard ratio (HR): 1.765; 95% confidence interval (CI): 1.008~3.090)], TNM stage (HR: 1.899; 95% CI: 1.023~3.525), EBV DNA (HR: 1.322; 95%CI: 1.087~1.607), lactate dehydrogenase level (LDH) (HR: 1.784; 95%CI: 1.032~3.086), high sensitivity C-reactive protein (hs-CRP) (HR: 1.840; 95%CI: 1.039~3.258), high-density lipoprotein cholesterol (HDL-C) (HR: 0.503; 95%CI: 0.282~0.896), hemoglobin (HGB) (HR: 0.539; 95%CI: 0.309~0.939) and lymphocyte to lymphocyte ratio (LMR) (HR:0.531; 95%CI: 0.293~0.962) were selected into the nomogram for survival. The C-index in the primary cohort and validation cohort were 0.800 and 0.831, respectively, which were statistically higher than the C-index values for TNM stage (0.672 and 0. 716), EBV DNA (0.668 and 0.688), and TNM stage+ EBV DNA (0. 732 and 0. 760), $p < 0.001$ for all. And the decision curve analyses showed that the nomogram model had a higher overall net benefit than the TNM staging system, EBV DNA and TNM stage+ EBV DNA. Then we stratify patients into three distinct risk groups for OS based on the total points (TPs) of nomogram: a low risk group (TPs ≤ 19.0), an intermediate risk group (19.0 < TPs ≤ 25.5) and a high risk group (TPs > 25.5), respectively. **Conclusion:** We have generated nomogram predicting prognosis for NPC patients with a higher predictive power than the TNM staging system, EBV DNA and TNM stage+ EBV DNA.

A-056

Molecular analysis of *MEN 1 gene* in suspected carriers of Multiple Endocrine Neoplasia type 1 born in Argentina

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Background: MEN 1 is an autosomal dominantly inherited syndrome characterized by parathyroid, gastroenteropancreatic and anterior pituitary tumors. Familial MEN1 (F) is defined in an individual who has at least one first-degree relative with one or more main endocrine tumors and Sporadic MEN1(S) when one individual is affected within a family with no history of the disease. The gene related to this syndrome is *MEN1*,it is a tumor suppressor gene located in chromosome 11q13 and codes for menin, a nuclear protein of 610 amino acids. Genetic diagnosis requires the sequencing of the whole DNA coding sequence and is warranted in patients with two or more of the characteristic tumors, their first-degree relatives, or young patients carriers one of

the tumors. AIM: To evaluate the *MEN1* germline mutations in patients with clinical features of MEN 1 born in Argentina. Subjects: We studied 127 potential carriers: 56 were index-cases (31 female and 25 male) and 71 first-degree relatives. The DNA of 66 healthy subjects was analyzed as a control group. All subjects gave informed consent to genetic studies. Methods: Genomic DNA was obtained from peripheral blood leukocytes. Coding region from the promoter to exon 10 and intronic flanking regions were amplified by PCR. The DNA fragments were sequenced after being manually labeled with ddNTP33 and since 2009 by automatic Sanger Sequencing. Long Range PCRs were performed in patients in whom no mutation was found and actually MLPA assay was evaluated. Pathogenic variants were confirmed in another DNA sample. Novel pathogenic variants were eventually confirmed by RFLP-PCR. Missense novel variants were verified by sequencing 104 alelles of a normal control population to evaluate the clinical significance. Results: We found germline mutations in 68% of index-cases: 55.3% were Familial MEN 1 and 44.7% were Sporadic MEN 1. Within the group of the Familial MEN 1 germline mutations were in 84% and 55.0% in the Sporadic MEN1 group. Germinal mutations were in the 38% of the first-degree relatives. The pattern of mutation type was: frameship(FS)(47%), missense(MS)(23.7%), nonsense(NS)(23.7%) and 5.3% of splice site(SS). The germline Variants/Exon found were:c.1060_1063dupTGCC/8;c.1340T>C/9,c.1102 delG/8,c.22A>T/2; c.471delG/3; c.487delG/3; c.791T>C/5, c.1350+1G>A/9, c.249-252delGTCT/2, c.1045C>T/7, c.551T>A/3, c.244delG/2, c.625_628delCAGA/3, c.1127T>C/8, c.1405G>T/10, c.378delG/2, c.672 delA/4, c.377G>A/2, c.828 C>G/6, c.1546_1547insC/10, c.784-9G>A/int4, c.1378C>T/10, c.1243 C>T/9, c.655 -1G>A/3, c.1102 del G/8, c.466 G>T/3, c.286 C>T/2, c.652 C>T/3, c.1243 C>T/9, c.483_495 del 13/3, c.1664G>T/10; c.792delC/5. The SNPs in our population were: c.1621G>A , c.512G>A, c.1254C>T, c435C>T, c.1080C>T and c.-533T>A. Conclusions: Germline mutations were detected in 67.9 % of MEN1 index cases and 38% in first-degree relatives. MEN1 mutations were distributed throughout the entire gene and included NS, FS, MS and SS mutations like other series that has been published. No correlation between phenotype and genotype was observed. The high frequency of novel mutations in the first series of patients with MEN1 born in Argentina and their presence in exons other than those reported in the literature it could be related to the ethnic and environmental factors from our population.

A-057

Agreement of Hevylite-based response assessment vs standard Electrophoresis/Immunofixation-based assessment: Follow-up of 26 Multiple myeloma patients

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Background: Protein electrophoresis (PE), immunofixation electrophoresis (IFE) and serum free light chains are the current gold standards for monitoring monoclonal proteins (MP). Precise evaluation of the changes on the concentration of MP is key to the management of Multiple Myeloma (MM) patients allowing clinicians to assess the success of the previously elected therapy. However, under certain conditions these techniques may present limitations rendering importance to the study of new biomarkers. **Objective:** to validate the use of the Hevylite® (The Binding Site) assay as a monitoring technique of MM patients by comparing it with the gold standard techniques. **Methods:** Quantification of monoclonal proteins in 26 MM patients during several courses of treatment and comparison of response criteria by Hevylite vs PE and IFE. The Hevylite assay quantifies immunoglobulins by their heavy/light chain pairs. Response assessment by Hevylite is based on the percentage of reduction of MP indicated by the International Myeloma Working Group (IMWG) criteria for PE/IFE. **Results:** 201 results obtained with Hevylite corresponding to a median of 10 (range: 6-19) determinations/patient. Response assessment based only on serum monoclonal evaluation (Table 1) showed an 83% overall agreement of Hevylite relative to SPE/IFE (only 1/172 samples showed more than 1 level of response difference). Hevylite identified all patients with progressive disease. Contingency analysis shows a progressive and statistically significant increase ($p<0.001$) in the number of patients with abnormal Hevylite Ig'k/Ig'ʎ ratio, as the quality of response decreases. Biochemical relapse is identified in 5 occasions by Hevylite and not by PE/IFE. Conversely, in 4 occasions IFE shows relapse from complete response while Hevylite does not. **Conclusion:** Long-term biochemical follow-up of the present cohorts shows good indications that Hevylite could work as a valid alternative assay for monitoring MM patients in case of limitations of the traditional techniques.

Table 1.Concordance of response assessment based on Hevylite vs PE/IFE

		PE/IFE assessment				
		MR	PR	VGPR	CR	
Hevylite assessment	MR (<50% MP decrease)	17	1	0	0	
	PR (>50% and <90%)	0	55	1	1	
	VGPR (>90% MP decrease)	0	7	17	5	
	CR (normal ratio)	0	0	14	54	
Agreement		**100%**	**87%**	**53%**	**90%**	**83%**

MR: minimal response, PR: partial response, VGPR: very good partial response, CR: complete response.

A-058

Development of a 2-Color biosensor-based ensemble FRET assay for discovery of potential substrates of the Cancer-implicated Human Multidrug Resistance Protein-1

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Human ATP-binding cassette (ABC) transporters are a superfamily of trans-membrane proteins responsible for efflux of drugs and other xenobiotics from the cell in an ATP dependent process. Overexpressed by cancer cells, Multidrug resistance-associated protein 1 (MRP1/ABCC1), a sub-member of ABC transporters, actively pumps out anticancer drugs, reducing the efficacy of the drugs and leading to failure of chemotherapy. The objective of the project is to identify potential substrates of MRP1 protein using a 2-color biosensor-based ensemble FRET technique. Since MRP1 affects the efficacy of drugs, profiling these drugs early for their MRP1-substrate status will inform the withdrawal of hit-drugs before they reach the more laborious and expensive clinical trials stages, thereby saving billions of dollars. This underscores the relevance of this project. Currently, there is no high throughput assay available to identify potential drug substrates of MRP1 protein. Previously, our group engineered a single 2-color MRP1 which reported intramolecular FRET as a function of structural changes and identified eight compounds as hits following a screening of NIH library of clinically tested compounds. The 2-color MRP1 construct was genetically modified by fusing a green fluorescent protein (GFP) and a red fluorescent protein (RFP) to the transporter. Here, we engineered a set of four functional 2-color MRP1 biosensors by switching the GFP positions to determine which GFP position improves FRET and investigate the constructs' interaction with the 8-compound hits using a steady state FRET-based assay. HEK 293 cells were transiently transfected with the 2-color constructs followed by confocal microscopy to observe the expression and localization of the proteins. Functional characterization of the 2-color biosensors was done through doxorubicin accumulation assay. 20 µg of each 2-color protein in tris sucrose buffer was prepared and incubated independently with 10 µM of the 8-test compounds and/or 4mM/5 mM ATP/$MgCl_2$ at 37 ^{0}C for 10 minutes prior to FRET Measurements using Fluorimeter model FL3-11. FRET efficiencies of compound conditions were normalized with that of apo condition. Fifty other drugs from an anti-cancer library were also screened by the same protocol using the most FRET sensitive construct, GR-888. 2-color MRP1 constructs GR-881, GR-888 and GR-905 demonstrated a change in FRET in the presence of meropenem, mesalamine and EGCG and/or ATP when compared to their ligand-free conditions. Changes in FRET by compounds alone were in some cases repressed by the addition of ATP suggesting biosensors could detect MRP1 modulators without ATP induction. Of the fifty anticancer drugs, ten hits showed percentage FRET change typical of MRP1 substrates. Overall, we have shown that except for GR-638, the 2-color MRP1 biosensors interact with potential MRP1 modulators and do hold promise for the discovery of novel MRP1 substrates using steady state FRET analysis. The long-term plan is to upscale this strategy to a high-throughput standard by using a regular plate reader to identify novel substrates of MRP1. These data, when published, are expected to have considerable impact on oncology.

A-059

Serum lactate dehydrogenase and C-reactive protein levels for the diagnosis of prostate cancer

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Background: Prostate cancer (PC) is a major health concern worldwide, being the second most common neoplasm and sixth cause of cancer-related death in the en-

tire world. Serum total prostate specific antigen (PSA) has become the most clinically useful tumour marker for the diagnosis and subsequent monitoring of PC. The free-to-total serum prostate specific antigen ratio (%fPSA) has been proposed to differentiate benign from malignant prostate disease in patients with nonspecific elevations of serum PSA levels (range between 4 and 10 ng/mL). Serum lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels can increase in many inflammatory processes and cancer. The aim of this study was to evaluate the utility of serum LDH and CRP levels for diagnosis of PC in men with nonspecific elevations of serum PSA levels.**Methods:** We studied men with no known history of PC and serum PSA levels between 4 and 10 ng/mL, who underwent 12-core transrectal ultrasound guided prostate biopsy. The following serum biomarkers were measured: PSA and free-PSA by electrochemiluminescence immunoassay on Hitachi Modular E-170 analyzer (Roche Diagnostics, Basel, Switzerland); LDH by enzymatic photometric method according to the International Federation of Clinical Chemistry and CRP by immunoturbidimetric test with monoclonal anti-CRP antibodies on Hitachi Modular cobas c 702 analyzer (Roche Diagnostics, Basel, Switzerland). The %fPSA was calculated using the following formula: (free-PSA/PSA)x100(%). Patients were classified into two groups according to the diagnosis of prostate biopsy: PC and NOT PC patients. Logistic regression was used for develop a probabilistic model to predict patients with PC and determine the importance of each biomarker by calculating the odds ratio. The diagnostic accuracy was determined using receiver operating characteristic curves (ROC), calculating the area under the ROC curve (AUC). **Results:** We studied 232 patients with ages between 43 and 98 years old (median=72), 200 NOT PC and 32 PC patients. Serum PSA and CRP levels were similar in the PC and NOT PC patients, in contrast serum LDH levels were higher in the PC patients and %fPSA values were higher in the NOT PC patients. Serum PSA and CRP levels were not statistically significantly to differentiate between PC and NOT PC patients ($p>0.05$). Serum LDH levels and %fPSA values were included in the probabilistic model to predict patients with PC. The odds ratios were 0.8530 and 1.0071 for %fPSA and LDH, respectively. The probabilistic model to predict patients with PC was: $(1+e^{-Z})^{-1}$; ($Z=0.0070xLDH-0.1589x\%fPSA-1.4898$). The AUCs were 0.657($p=0.0048$), 0.802($p<0.0001$), and 0.844($p<0.0001$) for serum LDH levels, %fPSA values and probabilistic model, respectively.**Conclusions:** Serum CRP levels were not useful to differentiate benign from malignant prostate disease, in contrast serum LDH levels could be used for diagnosis of PC in patients with serum PSA levels between 4 and 10 ng/mL. A probabilistic model to predict patients with PC using serum LDH levels and %fPSA values may improve the diagnostic accuracy compared to using %fPSA alone.

A-060

Fecal Immunochemical Test (FIT) Specimen Stability near the Clinical Cut-off

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Objective: The goal of this study was to investigate the stability of FIT specimens and to retrospectively assess potential patient impact of poor stability. **Background**: The Fecal Immunochemical Test (FIT) detects colonic bleeds, and is the most prevalent test for colorectal cancer screening across Canada. In Alberta, ≥75 ng hHb/mL is the clinical cut-off value used to reflex middle-aged patients for colonoscopy to search for neoplastic growth. FIT specimens are generally collected by the patient from home using a concealed, easy to use collection device. Patients are instructed to bring the collection to a laboratory site within 7 d following manufacturer claims of 14 d stability at ambient temperature. **Methods**: Specimens near the clinical cut-off (75 ng hHb/mL) values were considered to be most likely affected by instability or degradation, and thus were selected for this study. OC-Sensor Diana instrument (Somagen) ("give the address) was used in this study. Both QC material and patient specimens (n=9) were used in the following experiments. In experiment 1, FIT stability was assessed by selecting patient specimens (n=9) arriving to the laboratory on the date of home collection (day 0) that were initially 75-100 ng hHb/mL Specimens were stored at ambient temperature between measurements on 3, 5, 7 and 10 day post-collection. In experiment 2, specimens stored at ambient temperature for 7 and 14days post-collection (n=23) were tested. In experiment 3, retrospective analysis investigated turn-around time and the frequency of FIT results 50-100 ng hHb/mL obtained from laboratory information system (Cerner Millennium) under institutional data policies. **Results**: Within-day (using QC material) and within-run (serial measurement of patient specimens, n=9) precision were 13.5%, and 16%, respectively. In experiment 1, the pooled specimen mean on day 0 was 83.9 ± 7.3 ng hHb/mL, which consistently decreased with ambient storage. Indeed, the median percent decrease relative to day 0 was 16.5%, 24.6%, 46%, and 73.5% on d3, 5, 7, and 10, respectively. In experiment 2, 83% of specimens were initially positive (84 ± 13.1 ng hHb/mL). However, 7 d after collection and storage at ambient temperature, 26% of specimens remained positive; and after 14 d, only 16% of samples were above the clinical cut-off. Experiment 3 investigated 381,702 results reported from 2011-2017; and 87% were measured within 2d of collection. Cumulatively, 9.1% of patient results were ≥75 ng hHb/mL, and 1.7% were 75-100 ng hHb/mL. Importantly, 11,973 specimens were 'weak' negative (50-75 ng hHb/mL), of which, 2.5% were measured ≥5d post-collection. The potential patient impact was assessed in 2016 data from 3971 patients with results between 75-100 ng hHb/mL. 77% of those patients underwent colonoscopy, and colorectal cancer was diagnosed in 36 patients in this group (~1%), however, the presence of benign neoplasms requiring more intense follow-up could not be determined. **Conclusion:** Specimen stability is of great importance for the FIT testing system used in this study. Negative results in specimens stored at ambient temperature for more than 4 days should be repeated or interpreted with caution.

A-061

Reference Interval Determination and Method Comparison of the µTASWako and Beckman Access Total AFP Assays

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INTRODUCTION: Alpha-fetoprotein (AFP) can serve as a marker for a variety of tumors, including hepatocellular carcinoma, hepatoblastoma, and germ cell tumors. As a marker for hepatocellular carcinoma, AFP can be utilized either independently or as part of a L3-AFP index calculation [(AFP-L3 variant ÷ AFP Total) x 100%]. The µTASWako AFP-L3 assay (Wako Life Sciences, Inc.) instructions for use (IFU) lists reference intervals for the AFP-L3 assay, but no reference intervals are provided for the associated total AFP assay. As total AFP is often reported as part of an AFP-L3 index, this may lead to potential misinterpretation if no reference interval is given. **OBJECTIVE:** A method comparison was performed between the total AFP of the µTASWako AFP-L3 assay (Wako total AFP), and the Access total AFP assay (Beckman Coulter, Inc.; Beckman AFP). A reference interval determination was performed for both assays. **METHODS:** The reference interval study consisted of 140 apparently healthy individuals (120 adults and 20 pediatrics, evenly split by gender). These samples were collected from non-fasting patients, and the exclusion criteria included kidney disease, hepatic disease, autoimmune disorders, malignant neoplasms, pregnancy, and inflammatory bowel disease. A method comparison study was performed using these samples. Reference intervals at the 95th, 97.5th, and 99th percentiles were determined by non-parametric estimates. A separate method comparison was also performed using residual serum specimens (n =95) that extended a concentration range above the reference interval samples comparison. The analytical measuring range (AMR) of the Wako total AFP is 0.8 – 1000 ng/mL and is 0.5 – 3000 ng/mL for the Beckman AFP assay. **RESULTS:** The Passing Bablok regression fit analysis using the 140 reference interval specimens yielded the following total AFP equation: Wako total AFP (ng/mL) = 0.69 (Beckman AFP, ng/mL) - .0035 with a r^2 of 0.79. The data spanned ≤0.8 – 8.3 ng/ml for the Wako total AFP and 1.03 – 17.4 ng/mL for the Beckman AFP. Seventeen results were below the AMR of the Wako total AFP assay and were not included. The method comparison using the residual serum specimens (n=95) gave the following equation: Wako total AFP (ng/mL) = 0.99 Beckman AFP (ng/mL) - .3756 with a $r^2$of 0.99. This method comparison spanned 0.8 – 782 ng/ml (Wako AFP) and 1.24 – 877 ng/ml (Beckman AFP). The reference interval for Wako total AFP determined in the study was ≤3.3 at the 95th percentile, ≤3.4 at the 97.5th percentile, and ≤4.6 at the 99th percentile. The reference interval for the Beckman AFP determined in this study was ≤5.3 at the 95th percentile, ≤7.3 at the 97.5th percentile, and ≤8.3 at the 99th percentile. No significant differences were seen when partitioned by age or gender. The reference interval given in the Beckman IFU is <9.0 ng/mL (98.9th percentile, n=1126). **CONCLUSIONS:** While the methods agree well across the AMR, measured total AFP concentrations in the Wako assay differ significantly from the Beckman AFP assay in concentrations associated with healthy individuals. Reporting a separate reference interval for the Wako total AFP in the determination L3-AFP should be considered.

A-062

Serum YKL-40 as Biomarker for AFP-negative Hepatocellular Carcinoma

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Background: Alpha-fetoprotein (AFP) is the most widely used serum biomarker for hepatocellular carcinoma (HCC), despite its limitations. As complementary biomarkers, YKL-40, also called human Chitinase 3-like1, is a new biomarker for malignant tumor. Whether it may serve as a biomarker for AFP-

negative HCC remains unclear. This study aimed to investigate the usefulness of serum YKL-40 level as a biomarker for hepatocellular carcinoma (HCC). **Methods:** Enzyme linked immunosorbent assay was used to detect the serum YKL-40 level in 64 AFP-positive HCC patients ,93 AFP-negative HCC patients, 55 benign liver diseases (BLD) patients and 72 healthy controls (HC), respectively. The areas under the receiver operating characteristic(AUROC) curves of YKL-40, AFP and their combination were calculated and compared respectively. **Results:** It is showed that the optimal cut-off value to confirm a positive diagnosis of HCC was 69.48 ng/mL for YKL-40. The serum YKL-40 levels of all HCC patients (median 132.38 ng/mL, range 92.07-194.00) had significantly higher (P<0.001) than those patients with benign liver diseases (93.63 ng/mL, 57.94-136.22) or of a healthy control group (34.04 ng/mL, 18.10-59.40). The AUC of YKL-40 in AFP-negative HCC patients, AFP-positive HCC patients, and all HCC patients were 0.855 (95% CI, 0.804-0.905, P<0.01), 0.820 (95% CI, 0.756-0.884, P<0.01), and 0.841 (95% CI, 0.793-0.888, P<0.01), respectively. The sensitivity and specificity of YKL-40 for AFP-negative HCC patients, AFP-positive HCC patients, all HCC patients were 96.8% and 69.4%, 79.7% and 72.6%, 91.1% and 69.4%, respectively. When combining YKL-40 with AFP, the sensitivity and specificity were 96.8% and 72.6%, the AUC was 0.907 (95% CI, 0.873-0.942, P<0.01), which was statistically higher than that of AFP alone (AUC=0.862, 95% CI, 0.819-0.904, P<0.01). The results indicated that the diagnostic power improved significantly compared with either AFP or YKL-40 alone for HCC, and that YKL-40 had a better sensitivity for AFP-negative HCC diagnosis. **Conclusion:** Serum YKL-40 might be a better biomarker than AFP, and its combination with AFP may enhance the sensitivity of HCC. YKL-40 overexpression in serum had significant diagnostic power for AFP-negative HCC.

A-063

Diagnostic and high-grade cancer prediction performance of LDN-PSA glycosylation isoform

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Background: Although prostate specific antigen (PSA) is a widely used for prostate cancer (PCa) screening, the overdiagnosis and overtreatment of PCa due to low specificity of PSA is a major issue worldwide. To improve specificity, we focused PCa associated aberrant glycosylated PSA, LacdiNAc (LDN)-PSA, which has LacdiNAc structure on its *N*-glycan terminal. We have demonstrated a pilot study of LDN-PSA by automated immunoassay system which utilizes surface plasmon field-enhanced fluorescence spectroscopy (SPFS) as its detection principle. The aim of this study is to evaluate the diagnostic and high-grade PCa prediction performance of serum LDN-PSA directly compared to conventional PSA-based testing and indirectly compared PHI and PCA3 test. **Methods:** The serum LDN-PSA was measured by SPFS-based automated two step sandwich immunoassay system [Kaya, T. *et al.* Anal. Chem. 2015;87:1797-1803.]. Serum total and free PSA was tested on automated immunoassay analyzer Architect i1000 (Abbott Japan). All serum samples were collected before prostate biopsy (Pbx) and store at -80°C until use. To evaluate diagnostic and high-grade PCa prediction performance, a total of 528 patients with Pbx-proven benign prostatic hyperplasia (BPH, n=238) and Pbx-proven PCa (n=290) were enrolled. The grade group (GG) of Pbx specimens were evaluated according to the International Society of Urological Pathology guidelines. Predictive performance of each test was evaluated by ROC analysis. **Results:**Serum LDN-PSA levels in the range of total PSA <10 ng/mL and any PSA range were significantly higher in patients with PCa (median: 0.1175 U/mL and 0.2060 U/mL, respectively) than BPH (median: 0.0650 U/mL and 0.0670 U/mL, respectively), p<0.0001. At the cutoff LDN-PSA level (0.062 U/mL) for the prediction of PCa, the avoided biopsies rate was 45.4% at its 90% sensitivity, which was much higher than that of F/T ratio (32.5 %) and total PSA (18.8%). The AUC of LDN-PSA predicting PCa (0.8324; all range and 0.7462; <10ng/mL) was significantly higher than that of total PSA (0.7132 and 0.5715) and F/T ratio (0.7462 and 0.6899). Although, the patients background was different, we performed indirect comparison of diagnostic performance between LDN-PSA and FDA-approved markers. The diagnostic performance of LDN-PSA (AUC 0.74-0.83, NPV 82%, risk of missing PCa 10% and avoided biopsies rate 36-45%) is comparable to those reported value of PHI (AUC 0.70-0.77, NPV 67-92%, risk of missing PCa 8-33% and avoided biopsies rate 36%) and PCA3 (AUC 0.66-0.69, NPV 88-90%, risk of missing PCa 10-12% and avoided biopsies rate 44%). Serum LDN-PSA levels of PCa patients were much higher at Pbx GG 3 (median: 0.2100 U/mL) than Pbx GG 2 (median: 0.0915 U/mL), p=0.0001, while total PSA could not discriminate between Pbx GG 2 and 3. F/T ratio also much lower at Pbx GG 3 (median: 0.1765) than Pbx GG 2 (median: 0.3405), p=0.0004. The AUC of LDN-PSA predicting > Pbx GG3 (0.7947) showed quite better performance than that of total PSA (0.6005) and F/T ratio (0.7440). **Conclusion:**These data suggest that LDN-PSA improves diagnostic accuracy of PCa detection, which lead to large reduction of unnecessary biopsies. Predicting high-grade PCa patient by LDN-PSA could be used as a clinical index of patients under active surveillance.

A-064

Exosomal long non-coding RNA HOTTIP as a novel serum-based biomarker for diagnosis and prognosis of gastric cancer

X. Zhang, Y. Zhang. *Qilu Hospital of Shandong University, Jinan, China*

Objective: Long non-coding RNA HOTTIP plays important roles in the generation and progression of human cancers. Exosomes participate in cellular communication by transmitting molecular between cells and are regarded as suitable candidates for non-invasive diagnosis. However, the existence of HOTTIP in the circulating exosomes and the potential roles of exosomal HOTTIP in gastric cancer (GC) was poorly understood. This study aims to evaluate the existence of HOTTIP in the circulating exosomes and the potential roles of exosomal HOTTIP in GC. **Methods:** Exosomal HOTTIP was firstly detected in cell culture of GC SGC7901 cell line. Then, the stability of serum exosomal HOTTIP was evaluated by prolonged exposure to room temperature and treated with multiple freeze-thaw cycles. Finally, exosomal HOTTIP levels were detected by reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR) in sera of 246 subjects (126 GC patients and 120 healthy people). Receiver operating characteristic (ROC) curves and Cox analyses were used to evaluate its diagnosis and prognosis value, respectively. And the clinical value of exosomal HOTTIP were compared with traditional biomarkers, including CEA, CA 19-9 and CA 72-4. **Results:** Exosomal HOTTIP could be detected in culture medium of GC cell line, and the levels were increased with the incubation time extended. Exosomal HOTTIP were not affected after treated with prolonged exposure to room temperature or 3 freeze-thaw cycles. Levels of exosomal HOTTIP were also upregulated in GC patients than in normal controls (P < 0.001). And the levels were significantly correlated with invasion depth (P = 0.0298) and TNM stage (P < 0.001). The AUC for exosomal HOTTIP was 0.827, which demonstrated a higher diagnostic capability than CEA, CA 19-9 and CA72-4 (AUC = 0.653, 0.685 and 0.639, respectively) (P < 0.001). The Kaplan-Meier analysis showed a correlation between increased exosomal HOTTIP levels and poor overall survival (OS) (logrank P < 0.001), while no significant relationship was observed between OS and traditional tumors (CEA, CA 19-9 and CA72-4). And univariate and multivariate COX analysis revealed exosomal HOTTIP overexpression was an independent prognostic factor in GC patients (P = 0.027). **Conclusion:** Exosomal HOTTIP is directly released from GC cells, and may be a better biomarker for GC in diagnosis and prognosis than CEA, CA 19-9 and CA72-4.

Tuesday, July 31, 2018

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

Cardiac Markers

A-065

Association Of Single Nucleotide Polymorphisms (rs3798220) In The LPA Gene Region With Serum Lp(a) Levels In A Mixed Population

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Over the past several decades, numerous studies have established that increased levels of apolipoprotein(a) [Lp(a)] in plasma are associated with development of coronary heart disease (CHD). Upon discovery of the apo(a) gene (LPA), which was considered one of the most polymorphic transcribed genes in the human genome, researchers reported several polymorphism in LPA gene which associated with CHD and plasma Lp(a) levels. Recently, a single nucleotide polymorphism (SNP) rs3798220, also known as Ile4399Met, encoding an isoleucine to methionine substitution located in the protease-like domain of apo(a) at amino acid 4399 have been shown to be associated with CHD and plasma Lp(a) levels in Caucasians. This study investigated the association of SNP rs3798220 with plasma Lp(a) in a large scale of Berkeley HeartLab samples representing genetically diverse populations. The study showed that the heterozygous carriers of SNP rs3798220 (Ile/Met) had 2.8 fold higher serum Lp(a) levels with a mean of 64.3 mg/dL and 95% CI [63.1, 65.5] ($p = 0.0000$) compare to serum Lp(a) levels of homozygous non-carriers (Ile/Ile) having a mean of 33.4mg/dL and 95% CI [33.0, 33.6]. Interestingly, this study showed that the homozygous carriers (Met/Met) have 2.1 fold lower plasma Lp(a) than non-carriers (Ile/Ile) with a mean of 24.5mg/dL ($p = 0.0034$) and 6 fold lower than heterozygous carries (Ile/Met). This study also investigated the association of the same SNP with other biomarkers and concluded that there was a strong and clinically significant association between carriers of Ile/Met (genotype ag) and Met/Met (genotype gg) with high serum Triglyceride levels.

A-066

Study On Lipid Peroxidation &it Enzymatic Antioxidant Activities In Hypertensive Subjects

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Background
Lipid peroxidation is a degenerative process that affects cell membranes and other lipid containing structures under conditions of oxidative stress which is an essential early event in the pathogenesis of atherosclerosis as a major complication of hypertension. Hypertension is a major public health challenge; a major risk factor for cardiovascular disorder especially essential hypertension characterized by sustained elevation of blood pressure is without any identifiable cause. Hypertension is a major public health challenge; a major risk factor for cardiovascular disorder especially essential hypertension characterized by sustained elevation of blood pressure is without any identifiable cause. This study investigates the generation lipid peroxidation product (malonaldehyde) and its effects on physiological catalase, reduced glutathione and superoxide dismutase activities as enzymatic antioxidants.
Methods
The research subjects were selected from people in Ilesa metropolis of Osun State, Nigeria both male and female with age range of 31-60 years. The research subjects were grouped into two. Group one consist of 100 (male=50, female=50) newly diagnosed, untreated essential hypertensive subjects while group two consist of 100 non hypertensive subjects (male=50, female=50) that were not under any antihypertensive agent as control. Both systolic and diastolic blood pressures were determined using sphygmomanometer while the lipid peroxidation enzymatic antioxidant activities were determined using standard spectrophotometric techniques.
Results
There was a significant increase in serum concentration of MDA in hypertensive subjects ($p<0.05$) when compared with normotensive subjects. Also, serum level of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were significantly reduced in hypertensive subjects irrespective of gender ($p< 0.05$) when compared with normotensive subjects.
Conclusion
The significant increase in serum concentration of MDA in hypertensive subjects ($p<0.05$) suggest lipid peroxidative activity involvement in the etiology of hypertension. Serum level of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) significant reduction in hypertensive subjects irrespective of gender suggest their involvement in the neutralization of free radicals that has been linked with the pathogenesis of hypertension.

A-067

The NT-proBNP level in Subclinical stage of cardiac structural or functional abnormalities among health checkups

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Background: The heart failure stage B is defined as patients with abnormal heart structure/function without symptoms. Circulating concentration of NT-proBNP is raised in symptomatic patients with left ventricular (LV) dysfuction which caused by structural or functional Impairment. This study performed to investigate the association of NT-proBNP level with echo-defined cardiac structural or functional (diastolic) anomalies in asymptomatic subjects with preserved LV function (ejection fraction >50%).
Methods: We retrospectively studied 412 health examinees who underwent echocardiography and NT-proBNP test at a health promotion center in Seoul, between January 2016 and December 2016. Increased left ventricular mass index (LVMI), and left atrial dimension (LAD) were used as markers of structural anomalies, and septal e' velocity and E/e' were used as markers of diastolic dysfunction. NT-proBNP was measured by electrochemiluminescence immunoassay (Siemens Healthcare Diagnostics, DPC Immulite 2000 XPi, Tarrytown, NY, USA).
Results: Multivariate regression analysis indicated that the factors associated with higher NT-proBNP were older age, female sex, lower BMI, lower blood pressure, higher creatinine, and higher LAD. The NT-proBNP levels were higher with increasing age groups, lowest in those aged ≤45 years and highest in those aged >60 years ($P<0.001$). While female in those aged ≤60 years demonstrated higher NT-proBNP levels than males ($P<0.001$), there was no significant difference of NT-proBNP levels in those aged >60 years. The structural anomalies, which were defined increased LVMI or LAD, demonstrated higher NT-proBNP than normal LVMI and LAD ($P<0.05$). However, diastolic dysfunction, which was defined decreased septal e' velocity or increased E/e', was not associated with NT-proBNP level.
Conclusion: The level of NT-proBNP was associated with subclinical cardiac structural anomalies but not associated with diastolic dysfunction in asymptomatic health checkups.

A-068

Diagnostic performance of copeptin for acute myocardial infarction in emergency department

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Background: The aim of this study was to investigate the effectiveness of copeptin in the diagnosis of acute myocardial infarction (AMI), and to compare the diagnostic performance of copeptin with other cardiac markers.**Methods:** We prospectively enrolled 293 patients presenting with chest pain (onset within 12 hours) suggestive of acute coronary syndrome (ACS) to the emergency department. Serum CK-MB, troponin I and copeptin levels were measured in each patient and were compared between ACS groups for statistical differences. The accuracies troponin I, CK-MB and copeptin for AMI diagnosis were assessed by ROC curve analysis. The performance of each marker and combination of three markers is assessed by comparing their AUCs. And diagnostic performance of three markers was analyzed to onset of chest pain. **Results:** Median age was 60 years; 70.0 % were men; 24.6% were ultimately diagnosed with AMI. Patients were consisted of 41 ST segment elevation MI (STEMI), 31 non-ST elevation MI (NSTEMI), 87 unstable angina and 134 other diseases. The combination of three markers (AUC: 0.980, 95% CI: 0.957-0.993) had better diagnostic performance for AMI than troponin I (AUC: 0.801, 95% CI: 0.750-0.840) or CK-MB (AUC: 0.758, 95% CI: 0.705-0.806) or copeptin (AUC: 0.796. 95% CI: 0.745-0.840) alone ($P<0.001$). And the combination of three markers (AUC: 0.902, 95% CI: 0.862-0.934) had better diagnostic accuracy for STEMI than troponin I (AUC: 0.744, 95% CI: 0.690-0.793) or CK-MB (AUC: 0.676, 95% CI: 0.619-0.729) or copeptin (AUC: 0.844, 95% CI: 0.797-0.884) alone ($P<0.001$). The use of three

markers also showed superior performance for NSTEMI than troponin I (AUC: 0.779, 95% CI: 0.727-0.825) or CK-MB (AUC: 0.782, 95% CI: 0.730-0.828) or copeptin (AUC: 0.642, 95% CI: 584-0.697) alone. In patients with onset of chest pain less than 1 hour (1hr group), copeptin was most superior to other markers in diagnosis of AMI (AUC: copetin-0.739, CK-MB-0.620, troponin I-0.595, comparison of AUC: Copeptin vs CK-MB: P=0.028, Copeptin vs troponin I: P=0.003). In group with onset of chest pain less than 2 hours (2hr group), copeptin showed better performance than troponin I for AMI diagnosis (AUC: copetin-0.732, CK-MB-0.642, troponin I-0.609, comparison of AUC: Copeptin vs troponin I: P=0.009). The result of separating group showed that copeptin was best marker for early diagnosis to STEMI (comparison of AUC in 1hr group: Copeptin vs CK-MB: P<0.001, Copeptin vs troponin I: P=0.002/ comparison of AUC in 2hr group: Copeptin vs CK-MB: P<0.001, Copeptin vs troponin I: P<0.001). However, there was no difference in diagnostic performance according to onset of chest pain in NSTEMI group. And copeptin showed higher negative predictive value than other markers in STEMI patients (copeptin-96.71 (95% CI: 92.78-98.54), troponin I- 88.97 (95% CI: 86.85-90.79), CK-MB-89.39 (95% CI: 86.92-91.44).**Conclusion:** In chest pain patients, combination of copeptin in addition to troponin I and CK-MB improves AMI diagnostic performance. And copeptin especially helps in early diagnosis and rule-out of STEMI patients.

A-069

Early detection of doxorubicin-induced cardiotoxicity with high-sensitivity troponin T in chemotherapy-treated patients

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Background: Detection of chemotherapy-induced cardiotoxicity has historically relied on clinical presentation and cardiac imaging measures. Recently, global longitudinal peak systolic strain (GLS) measures with speckle tracking echocardiography (STE) and high-sensitivity troponin T (hs-TnT) have been utilized to evaluate the development of cardiotoxicity. The increased sensitivity of these methods may allow us to detect early development of cardiotoxicity and predict future cardiac dysfunction in chemotherapy-treated patients. We investigated the effectiveness of hs-TnT and GLS in detecting doxorubicin-induced cardiotoxicity. **Methods**: Thirty-six patients with newly diagnosed sarcoma were assigned to receive 72-hours doxorubicin infusion. hs-TnT was monitored before, and at 72 hours of each chemotherapy cycle. All samples were assayed at the same time using hs-TnT (Roche diagnostics). Elevated troponin was defined as hs-TnT > 5 ng/L. STE was performed pretreatment, after cycle 3, and end of chemotherapy. Only patients who received ≥ 150 mg/m^2 of doxorubicin and had at least two STE were included for evaluation of GLS and left ventricular ejection fraction (LVEF). **Results**: Six patients (25%) developed cardiotoxicity as defined as a decline in LVEF >10% by the Cardiac Review and Evaluation Committee. The absolute levels of hs-TnT had significantly peaked from precycle baseline, increased starting at cycle 2, subsequently in each precycle and during the cycle of therapy (p<0.05). Fold changes over baseline hs-TnT level were also significantly increased. In all six patients with cardiotoxicity, GLS increased significantly at the end of chemotherapy, compared with baseline (-21±2 vs -19±2). The increase in GLS by 15% and hs-TnT by 5ng/L were independent predictors of the development of cardiotoxicity at the end of chemotherapy (p<0.05). **Conclusion**: In conclusion, hsTnT and GLS predict the development of cardiotoxicity in patients treated with doxorubicin. These two parameters may be useful in predicting and detecting the development of chemotherapy-induced cardiotoxicity and thus reduction of the incidence of its associated morbidity and mortality.

A-070

Comparison of analytical outlier rates between Roche 4th and 5th generation Troponin T assays using both serum and plasma samples

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Background: Analytical outliers occur with most troponin methods and can adversely affect patient management. Because higher sensitivity troponin reagents result in more troponin values that exceed the 99th percentile value, it may be difficult to identify these analytical outliers. In this study, we compared the analytical outlier rates of the Roche 4th generation Troponin T STAT (cTnT Gen 4) and Roche 5th generation Troponin T STAT (cTnT Gen 5) assays using both serum and plasma samples. **Methods:**

Paired rapid clot serum tubes (RST) and plasma separator tubes (PST) (N=1426 pairs) were collected from hospital patients with orders for clinical cTnT testing. RST and PST samples were centrifuged for 3 minutes at 4000 x g and analyzed on cTnT Gen 4 and cTnT Gen 5 assays on a Roche Cobas e411 immunoassay system. If either of the paired samples displayed measurable cTnT Gen 5 (≥6 ng/L), both samples were stored at 2-8°C within 2 hours of initial measurement (N=1185 pairs). Within 24 hours of initial analysis, paired samples were warmed to room temperature, aliquoted, re-centrifuged at 4000 x g for 3 minutes, and re-analyzed on both Gen 4 and Gen 5 methods. We defined analytical outliers as:

• Initial and repeat results differing on the cTnT Gen 4 assay by >0.03 ng/dL for results <0.20 ng/mL or ≥20% for results ≥0.20 ng/mL

• Initial and repeat results differing on the cTnT Gen 5 assay by >10 ng/L for results <100 ng/L or ≥10% for results ≥100 ng/L

We also calculated the number/percent of repeat values that were on different sides of the 99th percentile upper reference limit (URL) for each assay. **Results:** Using the cTnT Gen 4 assay, 379/1426 (26.6%) RST and 391/1426 (27.4%) PST results were above the 99th percentile of ≥0.01 ng/mL. Using sex-specific 99th percentile cut-offs on the cTnT Gen 5 assay of > 10 ng/L (females) and >15 ng/L (males), 809/1426 (56.7%) RST and 802/1426 (56.2%) PST results were above 99th percentile. For cTnT Gen 4, 6/1185 (0.6%) RST and 8/1185 (0.7%) PST samples analyzed resulted in analytical outliers all of which were all at least 5 times above the 99th % URL. For cTnT Gen 5, we observed analytical outliers in 10/1185 (0.8%) RST and 10/1185 (0.8%) PST samples. However 15% of Gen 5 outliers had repeat values on different sides of the 99th percentile URL. For both Gen 4 and Gen 5 reagents, 50% of outliers had higher TnT results upon repeat testing while 50% had lower results. **Conclusion:** Analytical outliers occur frequently (0.5-1.0% of samples) with both Gen 4 and Gen 5 cTnT assays independent of sample type. No outlier results occurred on different sides of the 99th percentile URL for Gen 4. 5th Gen cTnT had a similar outlier rate but more were relevant to the determination of an elevated value. Compared to 4th Gen cTnT, use of 5th Gen cTnT will increase the percent of patients with elevated (above 99th percentile URL) values without reducing the rate of analytical outliers.

A-071

Comparison of the sensitivity and specificity of the RAMP® Troponin I assay and ADVIA Centaur® TnI-Ultra Assay

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Background: Measurement of cardiac troponin I (TnI) aids in the diagnosis of acute myocardial infarction (AMI) and in the prioritization of patient management. The purpose of this study was to compare the sensitivity and specificity of the RAMP® Troponin I assay on the RAMP 200 instrument and ADVIA Centaur® TnI-Ultra Assay on the ADVIA Centaur CP instrument[1]. The ADVIA Centaur CP System is a mid-volume, high throughput bench top laboratory instrument with chemiluminescent technology; the RAMP system is a lateral flow fluorescent immunoassay platform with a smaller footprint. Testing was performed over two days in the laboratory of a large urban hospital in Portland, OR. **Methods:** Paired lithium heparin plasma and EDTA whole blood waste samples were used for this study. Specimens were selected by medical laboratory staff based on the ADVIA Centaur CP Troponin I result listed in the laboratory information system, de-identified, and provided to study personnel. Lithium heparin plasma samples were retested on the ADVIA Centaur CP concurrently with EDTA blood samples on the RAMP system. Results were compared between instruments, and to the patient diagnosis as determined from the electronic medical record by medical staff. **Results:** EDTA RAMP results were compared to the original lithium heparin Centaur results. Retesting of lithium heparin specimens in the Centaur CP was not reliable due to the presence of fibrin clots in the original specimens. A total of 74 samples were included in this study; 2 samples were excluded due to specimen age (>12 hours elapsed since original testing) and 1 sample was excluded due to an error during sampling. The RAMP Troponin I and ADVIA Centaur TnI-Ultra Assay results showed 97% concordance. Using the 99th percentile as a cutoff, the RAMP Troponin I test (< 0.10 ng/mL) showed comparable sensitivity and specificity, 81% and 91% respectively, to the Advia Centaur TnI-Ultra Assay (< 0.04 ng/mL), 75% and 91% respectively, when compared to the electronic medical record (i.e. ECG result). Also comparable were the positive predictive values (PPV), 84% and 84%, and negative predictive values (NPV), 89% and 85%, for the RAMP and Centaur systems respectively. **Conclusion:** The possibility of using the RAMP Troponin I test at immediate and urgent care facilities is very attractive. Both the RAMP Troponin I test

and the ADVIA Centaur TnI-Ultra Assay showed excellent specificity, when used in conjunction with other clinical findings (e.g. abnormal ECG), in the diagnosis (rule-in) of AMI. The results of the study therefore support the use of the RAMP Troponin I test on the RAMP 200 system as an alternative to the larger laboratory systems, where maintenance and calibration downtime, limited space, or lower volumes would necessitate a smaller, yet effective option. [1] Both devices are available for sale in the US and are CE Marked.

A-072

Single Molecule Technology: Equivalence between a research platform and a CE-marked diagnostic platform for the quantification of cardiac troponin I

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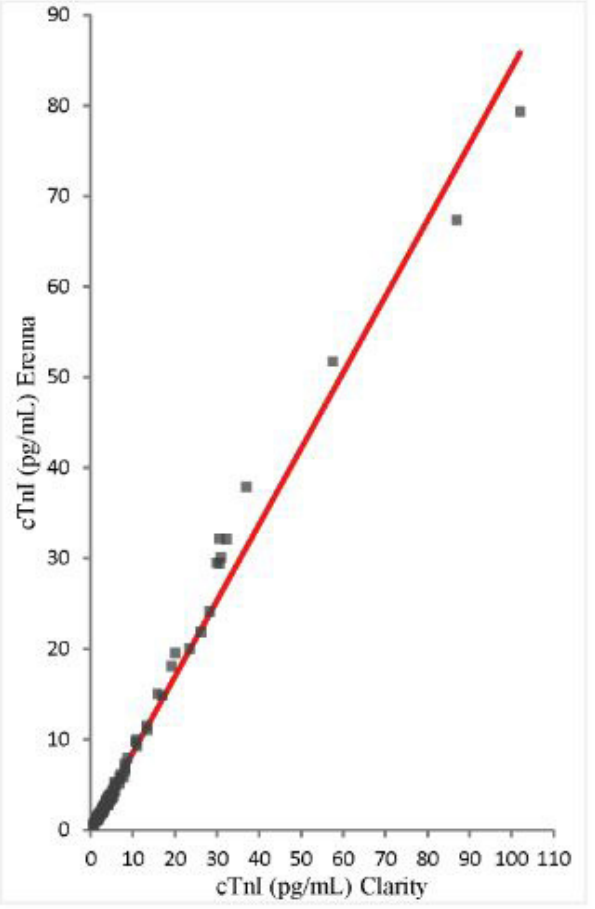
Background: Single Molecule Counting technology has enabled the quantification of intractable low-abundance biomarkers. The Erenna® Instrument (research use only, RUO) and the CE-marked diagnostic Singulex Clarity® system are powered by Single Molecule Technology. Numerous studies have generated clinically important information on RUO-based platforms, but their translatability to clinically useful platforms has not been demonstrated. In this study, the correlation and equivalence of the Singulex Clarity system and the Erenna Instrument for measurements of cardiac troponin I (cTnI) were evaluated. **Methods:** De-identified EDTA-plasma samples (n = 120) were first tested by the Singulex Clarity® cTnI assay on the Singulex Clarity system (limit of quantification 0.14 pg/mL) and subsequently measured on the Erenna Instrument. The study on frozen samples, biobanked from a CLIA-licensed clinical lab, were selected to span a wide range of the assay. Passing-Bablok and Pearson's R correlation analyses were performed to compare and quantify the linear relationship between the assays. **Results:** cTnI was measured in all samples and the concentrations ranged from 0.54 to 102.03 pg/mL, as measured by the Singulex Clarity system. When comparing the Singulex Clarity system and the Erenna Instrument, the Pearson correlation coefficient was 0.99 (95% CI: 0.99-1.00; Figure), indicating nearly all the variance in the Singulex Clarity results could be explained by the Erenna results. The coefficient from the Passing-Bablok regression was 0.85 (95% CI: 0.82-0.89), indicating a slight bias between the two instruments that may be explained by standardization differences. **Conclusion:** The Singulex Clarity cTnI assay on the Singulex Clarity system had good correlation with the Erenna Instrument for cTnI measurements in EDTA plasma, as indicated by a strong linearity relationship between the platforms. The systems provided substantially equivalent results, demonstrating that findings on cTnI measurements using the Erenna Instrument are equivalent to those obtained using the Singulex Clarity system.



A-073

Performance Evaluation of Atellica IM High-Sensitivity Troponin I Assay in a Clinical Chemistry Laboratory

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Background: Cardiac troponins have become the preferred biomarker for diagnosis of MI. As sensitivity of troponin assays has increased, so has the precision at the lower end, shortening time points between serial measurements, and improving the sensitivity for early detection of MI. The Atellica® IM High-Sensitivity Troponin I (TnIH) Assay* is an in vitro diagnostic immunoassay for the quantitative determination of cardiac troponin I in serum or plasma. The objective of this study was to verify the analytical performance (precision and linearity) of the Siemens Healthineers Atellica IM TnIH Assay on the Atellica® IM 1600 Analyzer, and perform method comparison with the ADVIA Centaur® High-Sensitivity Troponin I (TNIH) Assay. (*Not available for sale in the U.S. Future availability cannot be guaranteed.) **Methods:** The Atellica IM TnIH Assay is a dual-capture sandwich immunoassay using magnetic latex particles, a proprietary acridinium ester for chemiluminescence detection, and three monoclonal antibodies. The precision studies were evaluated according to EP05-A3 and EP15-A2 and method comparison to EP09-A3. Precision studies used lithium heparin plasma samples, two sample pools, and three levels of controls. One aliquot of each sample pool and each QC material was tested in replicate in two runs per day on each analyzer for a minimum of ten days with one lot of reagent and calibrator. Each run was separated by approximately a two-hour time interval. A total minimum of 40 replicates were generated per sample. Serial measurements were obtained for lithium heparin samples from >50 chest pain Emergency Department patients. Troponin samples at admission and 1, or 2, or 3, or up to 6 h later were analyzed using the Atellica IM TnIH

Assay, and the ADVIA Centaur TNIH assay. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. **Results:** Precision studies agreed with the manufacturer's claims: Within day CV%(SD)s were 5.4(0.61), 4.1(1.04), 2.1(0.79), 1.9(67.33), 1.8(325.83) for concentrations of 11.4, 25.4, 37.1, 3497.2, 18056.4 ng/L (pg/mL); within lab (total) CV%(SD)s were 6.9(0.79), 4.4(1.12), 3.4(1.27), 3.0(104.48), 2.4(430.79), respectively. Method comparison between the Atellica IM TnIH Assay and ADVIA Centaur® High-Sensitivity Troponin I (TNIH) assay showed a regression slope of 1.045 (95%CI 1.03 to 1.06), intercept of -2.396 pg/mL(95%CI -2.62 to -2.00) (n=77). Serial measurement results demonstrated 100% total agreement for subjects falling above and below the respective assay 99th percentile value, when comparing Atellica IM TnIH Assay with ADVIA Centaur TNIH assay. **Conclusion:** The Atellica IM TnIH Assay has demonstrated good precision for detecting low cardiac troponin I concentrations, good correlation and agreement with the Siemens ADVIA Centaur TNIH assay.

A-074

assessment of plasma hepcidin concentration as a novel biomarkers of acute coronary syndrome severity

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Background: Hepcidin, produced mainly by liver hepatocytes, is the principal systemic iron regulator. Hepcidin is an acute phase reactant that plays a role in the progression of inflammatory caused diseases including thrombosis formation in coronary artery diseases (CAD). We assessed serum hepcidin level in CAD patients with acute coronary syndrome (ACS). The association of classical atherosclerotic markers such as cardiac troponin I (cTnI), and C reactive protein (CRP) was compared to hepcidin and ferritin serum levels. **Methods:** A total of 80 subjects (60 ACS patients and 20 controls) were enrolled. Sera of ACS patients admitted at the Emergency Department were obtained within 6 hours of chest pain. ACS patients were subdivided into: unstable angina (UA,n=20), non-ST elevation myocardial infarction (NSTEMI, n=20), and ST elevation myocardial infarction (STEMI n=20). Serum hepcidin and ferritin were evaluated using enzyme-linked immunosorbent assay (ELISA). CRP, cTnI, and lipids were measured using spectrophotmetry. The results were statistically compared. **Results:** ACS patients were 68.8% males and 31.3% females. Their overall mean age was 56.00±8.73. Healthy controls mean age was 50.2±9.03 and included 13/65% males and 7/ 35% females. 53.1% of ACS patients were hypertensive and 39.1% were diabetics compared to 15% hypertensive and 10% diabetics in the healthy control group. ACS patients have higher TG, LDL and lower HDL (means114.3±47.6, 127.0±45.2, 35.8±9.3 mg/dl respectively) compared to controls. Mean random blood glucose was 174.4 ±68.2 in ACS and 111.3±30.0 in control subjects. While hepcidin was nearly three folds higher in STEMI patients compared to control, mean 44.5±21.0 and 16.6±17.9 ng/ml respectively (P<0.001), NSTEMI and UA showed near control hepcidin levels. The increased hepcidin in STEMI patients accompanied a significant low level of ferritin compared to healthy control (47.4±41.3 and 118.1±99.9 ng/ml respectively). STEMI, NSTEMI and UA had similar pattern for both Troponin I and CRP plasma levels, the three ACS clinical classification had significant higher Troponin I (9.94±13.19, 5.30±5.75, 0.01±0.02 ng/mL respectively) and CRP (14.1±13.43, 13.2 ±18.9, 6.9±7.3 mg/l respectively) compared to control levels 0.002±0.003ng/ml and 1.4 ±0.3mg/l troponin I and CRP respectively. In the present study, ACS patients had a significant positive correlation between hepcidin and each of troponin I and CRP. The results suggest that hepcidin levels increase with increasing ACS disease severity. Therefore, hepcidin levels may be a useful marker to follow progression of ACS. As the mean iron regulating hormone, in principle, hepcidin is an excellent therapeutic target for strategies to reduce the inflammation associated with the generation of atherosclerosis in ACS. **Conclusion:** We concluded that serum hepcidin is increased in STEMI compared to NSTEMI, UA. The increased hepcidin accompanied a high level of cardiac troponin I and CRP only in STEMI patients but not the NSTEMI. Our findings highlight the association of hepcidin and ACS progression. The present study may provide a preliminary basis for broader scale studies to highlight the interaction of hepcidin with various inflammatory players involved in atheroma formation as well as the underlying activated signaling pathways to explain the mechanisms for hepcidin release in CAD. **Key words: ACS, Hepcidin, Ferritin, STEMI, NSTEMI, UA.**

A-075

Performance of Emergency Testing Functionalities for Atellica® IM TnIH, hCG and BNP Assays on the Atellica® Solution

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Background: The objective of this study was to verify the STAT capabilities of the Atellica Solution, consisting of an Atellica® Sample Handler and two Atellica® IM 1600 Analyzers, with two metrics: (1) turnaround time (TAT) for emergency STAT tests while simultaneously performing routine testing and (2) impact of the STAT capabilities of the system on the TAT of the emergency samples. **Methods:** The study reproduced a typical 3 hour peak period of the day for 650 routine samples with 1561 test requests representative of the lab's workload. To include STAT testing, normally done in a dedicated laboratory, we added to the worklist a representative day's quantity of High-Sensitivity troponin I (TnIH), total hCG (hCG), and B-type natriuretic peptide (BNP) STAT tests corresponding to those same 3 hours, thus creating a single worklist including both routine and STAT. Hence, we were able to load STAT samples into the recreated peak routine testing and observe TAT from tube scanning on the Atellica Solution to result delivery. Routine samples were loaded in 10 minute intervals; STAT tubes were loaded according to the timestamps collected from the original STAT laboratory data. Since hCG was run both as routine and STAT we were able to measure the impact of the system's STAT functionalities on TAT. **Results:** TAT of STAT assays:

	As STAT				As routine			
Assay	**Samples**	**Mean TAT (min)**	**TAT CV (%)**	**TAT range (min)**	**Samples**	**Mean TAT (min)**	**TAT CV (%)**	**TAT range (mins)**
TnIH	13	10.9	6	10.5-12.7	N/A	N/A	N/A	N/A
hCG	6	10.9	4	10.5-11.6	10	11.9	12	10.5-14.8
BNP	7	11.1	6	10.5 – 12.5	N/A	N/A	N/A	N/A

N/A: Not applicable

Conclusion: The Atellica Solution is able to deliver results with a quick and predictable TAT for STAT assays, including TnIH, hCG, and BNP, while simultaneously performing routine testing with minimal impact on throughput. Furthermore, comparing TAT of hCG in STAT vs. routine shows that the Atellica Solution's STAT capability effectively reduces TAT and variability.

* Siemens Healthineers supported the study by providing systems, reagents, protocols and contributed to data analysis

A-076

High-Sensitivity Cardiac Troponin I Whole Blood and Plasma Specimen Comparisons Measured by the ET Healthcare Pylon Point of Care Assay

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Introduction: Cardiac troponin (cTn) testing is the guideline recommended biomarker for ruling in and ruling out acute myocardial infarction (MI) and risk stratification of patients presenting to emergency departments with ischemic symptoms. High sensitivity (hs)- cTnI assays are transitioning to become the optimal methods because of improved analytical performance. The objectives of our study were to compare a) POC hs-cTnI concentrations between matched whole blood and plasma specimens from 40 patients admitted through the emergency department using a novel point of care (POC) assay and b) plasma POC hs-cTnI concentrations with plasma measured on central laboratory hs-cTnI and Gen 5 cTnT assays. Methods: Fresh, waste whole blood EDTA anticoagulated specimens (n=40) were collected in the emergency department. Within 2 hours, the whole blood specimens were analyzed, and then immediately centrifuged to separate the EDTA plasma, which were than immediately analyzed. Whole blood and plasma measurements were performed on the novel Pylon hs-cTnI assay by ET Healthcare; currently only cleared for patient use in China. Further, the POC Pylon plasma results were compared with an investigational hs-cTnI assay by Abbott (ARCHITECT i1000) and the Roche Gen 5 cTnT assay (cobas e601). Specimens were enrolled over a 5-day period. Results: 98% of whole blood and 100% of plasma specimens were measureable

by the Pylon; ranges: whole blood 1.1-61 ng/L; plasma 1.4 to 59, ng/L. The correlation between whole blood and plasma showed the following: WB hs-cTnI = 0.98 plasma hs-cTnI + 1.01. The plasma correlations measured on the Pylon and the a) ARCHITECT and b) cobas e601 showed the following: Pylon hs-cTnI = 0.32 ARCHITECT hs-cTnI + 7.85; Pylon hs-cTnI = 0.10 cobas hs-cTnT + 11.05; respectively. The plasma correlation between the ARCHITECT and cobas e601 showed the following: ARCHITECT hs-cTnI = 0.26 cobas hs-cTnT + 11.56. Conclusions: Preliminary findings of the POC Pylon ET Healthcare hs-cTnI assay showed excellent agreement between whole blood and plasma. Correlation between the Pylon hs-cTnI and ARCHITECT hs-cTnI assays was excellent for 36 of the 40 plasma samples studied; 4 samples showed higher results on the Pylon than the ARCHITECT, resulting in a decrease in the overall correlation. Correlation of cobas hs-cTnT with both the Pylon and the ARCHITECT was poor. Additional studies are underway to evaluate the clinical performance of this POC hs-cTnI assay.

A-077

Method Comparison of 5th Generation "High-Sensitivity" Troponin T with 4th Generation Troponin T

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Background: The Food and Drug Administration cleared Roche Diagnostics Elecsys Troponin T Gen 5 STAT (gen5 cTnT) assay in January 2017, making it the first next generation troponin assay available in the United States. The assay was implemented at our hospital to assist with the rapid rule out of acute myocardial infarction (AMI) and risk stratification of acute coronary syndromes. Analytical relationships were explored between gen5 cTnT and Troponin T STAT (gen4 cTnT; Roche Diagnostics), with emphasis near lower limits of measure. Further, the association between plasma creatinine concentration and gen5 cTnT result was evaluated. **Methods**: Comparisons were made between the gen5 cTnT and gen4 cTnT assays on 4 Cobas 8000 e602 (Roche Diagnostics) instruments. All non-less than gen4 cTnT (>0.09 ng/mL) and gen5 cTnT (>5 ng/L) results were plotted, and Deming regression analysis was performed. Further, additional comparisons were made between plasma creatinine concentration and gen5 cTnT. Gen5 cTnT results >51 ng/L were excluded from analysis and <6 ng/L results were included as 6 ng/L. The comparisons were made between the averaged gen5 cTnT result per group and the grouped plasma creatinine concentration: 0.60-0.79, 0.80-0.99, 1.00-1.19, 1.20-1.39, 1.40-1.59, 1.60-1.79, and 1.80-1.99 mg/dL. Data for all studies was collected from 07/05/17 to 12/03/18. **Results**: The analysis included 614 points from a gen5 cTnT (y axis) range of 6 to 1391 ng/L and a gen4 cTnT (x axis) range of 0.010 to 1.360 ng/mL. The regression analysis displayed a Pearson coefficient (R) of 0.9845, a slope of 901, and intercept of 24. Deming regression analysis was also performed in a smaller sub range, focused on the lower measuring range (i.e. closer to clinically important thresholds). The analysis plotted 373 points from a gen5 cTnT (y axis) range of 6 to 82 ng/L against a gen4 cTnT (x axis) range of 0.010 to 0.050 ng/L. The regression analysis displayed an R of 0.7806, a slope of 1490, and an intercept of 11. The sub range displays a lower correlation compared to the larger range and a proportional bias. To further evaluate differences in the methods, gen4 cTnT results <0.010 ng/mL were compared to the gen5 cTnT results. Of the 3409 samples that were <0.010 ng/mL on the gen4 cTnT assay, 1224 and 1935 results were <6 ng/L or <10 ng/L, respectively, on the gen5 cTnT assay. The remaining 1473 gen5 cTnT results ranged from 10 to 45 ng/L. Linear regression analysis was performed, comparing the averaged gen5 cTnT result to its corresponding creatinine concentration group, displaying an R of 0.987. The lowest plasma creatinine concentration group (0.60-0.79 mg/dL) yielded an average gen5 cTnT of 9 ng/L, and the highest plasma creatinine concentration group (1.80-1.99 mg/dL) gave an average gen5 cTnT of 27 ng/L. **Conclusion**: Overall, gen5 cTnT has a strong linear relationship versus its predecessor assay, gen4 cTnT. However, the correlation decreases towards their respective lower limits. Gen5 cTnT also displays a strong relationship to plasma creatinine concentration.

A-078

NT-proBNP assays that are based on antibodies which are specific to nonglycosylated regions of NT-proBNP display a similar diagnostic accuracy in distinguishing heart failure patients compared to the Roche NT-proBNP assay

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Background: N-terminal fragment of pro-B-type natriuretic peptide (NT-proBNP) is a useful blood biomarker for the diagnosis of heart failure (HF). NT-proBNP is O-glycosylated within the central part and present in the circulation as a pool of molecules with different glycosylation levels. An automated NT-proBNP immunoassay manufactured by Roche is widely used for NT-proBNP measurements. This assay employs monoclonal antibodies (mAbs) that are specific to the epitopes 27-31 and 42-46 in the central region of NTproBNP. One of the mAbs is specific to the partially glycosylated region of NT-proBNP as the epitope 42-46 comprises Ser_{44}, which is modified by glycosidic residues. The presence of O-glycans at this site makes NT-proBNP undetectable by the Roche NT-proBNP assay due to the steric hindrance. In light of this, the assay is able to detect only the NT-proBNP fraction that is nonglycosylated at the 42-46 region and not the "total" NT-proBNP, i.e. both glycosylated and nonglycosylated subfractions. Since O-glycosylation tends to be heterogeneous, its pattern and extent might vary significantly among individuals and this could in turn impact the clinical value of NT-proBNP measurements by glycosylation-sensitive NT-proBNP assays. We have developed an alternative type of NT-proBNP immunoassays that are not affected by analyte glycosylation and are able to measure the concentration of the "total" NT-proBNP. The aim of this study was to compare the diagnostic accuracy of measurements of the "total" NT-proBNP (by two prototype immunoassays) with measurements of NT-proBNP that is nonglycosylated at Ser_{44} subfraction of NT-proBNP (by the Roche NT-proBNP assay) in distinguishing HF from non-HF patients. **Methods:** NT-proBNP levels were measured by two HyTest's prototype NT-proBNP assays (capture mAb - detection mAb: $29D12_{5-12}$ - $NT34_{25-32}$ and $15C4_{67-73}$ - $13G12_{15-20}$) and the Roche NT-proBNP assay (automated Roche Cobas e 411 analyzer) in EDTA-plasma samples that were obtained from 51 patients who had been diagnosed with HF and 53 healthy individuals (age-matched). HyTest's prototype NT-proBNP assays were linear in the range of 20 to 80,000 ng/L and the detection limits were 5-10 ng/L. Recombinant nonglycosylated NT-proBNP 1-76 (HyTest, produced in *E. coli*) was used as a calibrator in the prototype NT-proBNP assays. The diagnostic accuracy of the assays was analyzed by the comparison of the ROC curves. **Results:** ROC-AUC for the prototype assays $29D12_{5-12}$ - $NT34_{25-32}$/$15C4_{67-73}$ - $13G12_{15-20}$ were 0.951/0.946 (sensitivity 0.86/0.84 and specificity 0.93/0.98 respectively) compared to 0.965 (sensitivity 0.86 and specificity 0.98) for the Roche NT-proBNP assay. Differences were statistically insignificant (p-value = 0.365/0.369). **Conclusion:** NT-proBNP immunoassays that are based on antibodies which are specific to nonglycosylated regions of the NT-proBNP molecule are expected to have at least a similar clinical value for HF diagnosis as the Roche NT-proBNP assay that detects only a subfraction of endogenous NT-proBNP. Taking into account the known high variability in levels and site occupancy of O-glycosylated proteins, we suggest that immunoassays which measure "total" NT-proBNP levels might be advantageous for HF diagnostics and/or therapy monitoring in certain groups of patients and disease states due to their ability to detect endogenous NT-proBNP independently of its glycosylation status.

A-079

Do High-Sensitivity Cardiac Troponin I Clinical Performance Data in Package Inserts Reflect Realistic Clinical Expectations?

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Background: Cardiac troponin (cTn) is a cornerstone for diagnosis and management of myocardial infarction (MI). High-sensitivity troponin (hs-Tn) provides earlier MI rule-in/rule-out. The 2015 European Society of Cardiology guidelines proposed hs-Tn algorithms for NSTEMI-management. However, estimates of hs-cTn performance may vary based on the anchor-time used for analysis. Typically cTn data have been organized relative to the "first study sample" (1stSS) collection time, including in

manufacturers' package inserts. Alternatively data can be organized based on time of presentation (TOP). We investigated diagnostic performance of the ADVIA Centaur® hs-TnI (TNIH) up to 3.5 hours using TOP or 1stSS anchor-time.**Method:** Samples from >2,300 'all-comer' suspected MI patients were collected at 29 IRB-approved sites; 310 (13%) patients were adjudicated MI positive. The TNIH assay was validated as hs-TnI: total CV was 2.9% at the female 99th percentile (37 ng/L); using the AACC Universal Sample bank, 58.9% and 85.8% of values from healthy women and men, respectively, exceeded LoD (1.6ng/L).**Results:** Median delay between local standard-of-care first blood draw and 1stSS was 49 min. Table-Section A shows TNIH sex-specific performance based on TOP analysis (n=4,502 observations). Table-Section B displays sex-specific performance using 1stSS (n=6,346 observations). At 3.5-hours, TOP sensitivity was 95.8% & 89.7% and Negative Predictive Value (NPV) was 99.5% & 98.1% for women and men, respectively. For 1stSS at 3.5-hours, sensitivity was 95.0% & 89.2% and NPV was 99.3% & 97.6% for women and men, respectively. Although at 3.5-hours, TOP (n=198) had more adjudicated MIs than 1stSS (n=114) (p<0.001), sensitivity/NPV was not significantly different than anchoring at 1stSS (p-value=0.44).**Conclusion:** Reporting performance relative to TOP or the 1stSS does not yield different values for sensitivity/NPV or other MI diagnostic parameters at 3.5 hours. We advocate reporting data anchored to clinical presentation time to facilitate harmonizing with clinical guidelines.

		Sensitivity		Specificity		PPV		NPV	
Sex	Time-point	N		N		N		N	
SECTION A- Anchored To Time Of Presentation (TOP)									
Females (cutoff=37 ng/L)	0 to 1.5 hr*	41	82.9%	396	93.9%	58	58.6%	379	98.2%
	1.5 to 2.5 hr*	76	89.5%	710	91.8%	126	54.0%	660	98.8%
	2.5 to 3.5 hr*	72	95.8%	605	91.9%	118	58.5%	559	99.5%
Males (cutoff=57 ng/L)	0 to 1.5 hr*	100	74.0%	561	92.0%	119	62.2%	542	95.2%
	1.5 to 2.5 hr*	162	87.7%	913	90.7%	227	62.6%	848	97.6%
	2.5 to 3.5 hr*	126	89.7%	740	90.1%	186	60.8%	680	98.1%
*from time of presentation									
SECTION B-Anchored To Time Of First Study Sample (1stSS)									
Females (cutoff=37 ng/L)	Baseline‡	102	87.3%	887	91.4%	165	53.9%	824	98.4%
	0.75 - 1.5 hr†	89	89.9%	820	91.6%	149	53.7%	760	98.8%
	1.5 - 2.5 hr†	44	97.7%	427	92.3%	76	56.6%	395	99.7%
	2.5 - 3.5 hr†	40	95.0%	317	87.4%	78	48.7%	279	99.3%
Males (cutoff=57 ng/L)	Baseline	194	81.4%	1088	90.9%	257	61.5%	1025	96.5%
	0.75 - 1.5 hr†	166	86.7%	1038	90.9%	238	60.5%	966	97.7%
	1.5 - 2.5 hr†	93	86.0%	605	90.6%	137	58.4%	561	97.7%
	2.5 - 3.5 hr†	74	89.2%	362	91.4%	97	68.0%	339	97.6%

‡baseline defined as study samples collected within +/-90 minutes from the time of the first local standard-of-care blood draw
†from the time of the 1st study sample

A-080

Macrotroponin T causing a false positive troponin elevation

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Presentation: A 54 year old Asian British male was admitted with a 4 day history of chest pain for 7 days. Past medical history: Chronic hepatitis B (e-Antigen negative), non-alcoholic steatohepatitis, hypertension and type-2 diabetes. Previous admissions for chest pain 4 and 2 years prior to this episode. Family history: Type 2 diabetes, hypertension and hypercholesterolaemia. Clinical course: Troponin T (cTnT) Roche Diagnostics Cobas 8000 (10% CV = 13ng/L, 99th percentile URL = 14ng/L) was elevated on admission and noted to remain consistently elevated. Investigations: Imaging by thoracic CT (aortic imaging, no abnormality detected), coronary angiography (no obstructive epicardial disease) and cardiac MRI (no evidence of injury or wall motion abnormality) did not support acute myocardial injury as a cause for the raised troponin. An analytical interference was suspected and serial dilution and polyethylene glycol (PEG) precipitation of the sample was performed and the sample was analysed for cardiac troponin I (Abbott diagnostics hs cTnI, 10% CV = 4.7 ng/L, 99th percentile URL = 26.2 ng/L). Results: cTnI was < 2ng/L. Serial dilution showed comparable recovery with a known native high troponin sample (y = 0.9856x - 60.759, R^2 = 0.9986). PEG precipitation showed a large disparity in recovery between original measurements and against known native troponin samples (Day 4 = 1.13% and Day 5= 1.36% recovery, control samples = 94.46% and 85.09% recovery). Conclusion: The results were consistent with macrotroponin and not acute cardiac injury. Macrotroponin T has not been widely reported.

Date	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3	Day 4	Day 5	Day 6
Troponin T (ng/L)	1588	1842	1690	1789	1684	1718	1745	1697	1674

A-081

First High-Sensitivity Cardiac Troponin I Assay Cleared by the United States Food and Drug Administration

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Background: High-Sensitivity (hs) cardiac troponin (Tn) assays must meet two criteria according to 2018 AACC Academy and IFCC TFCACB Expert Opinion Recommendations: (i) cTn values above the limit of detection (LoD) for >50% of male and female healthy cohorts of ≥300 individuals each; (ii) imprecision ≤10% total CV for sex-specific 99th-percentile clinical decision values (CDVs). No current FDA-cleared assay has evidence of hs-Tn criteria to date. The LoD, percent healthy females and males exceeding LoD, and total %CV at sex-specific CDVs were determined to examine hs-TnI status of the PATHFAST cTnI-II (TnI-II) assay. **Methods:** CLSI EP17 was used to determine the TnI-II's Limit of Blank (LoB) by LoB=mean+1.645xSD and the LoD by LoD=LoB+[1.645xSD]. CLSI EP15 was used for verifying TnI-II's total %CV at the 99th percentile CDV of the female, male and overall healthy populations. Lithium-heparin samples from the AACC Universal Sample Bank (USB), comprised of 847 healthy women (49.4%) and men (50.6%) was used to determine sex-specific 99th-percentile CDVs with Non-parametric, Harrell-Davis and Robust modeling. USB subjects with evidence of underlying health conditions from Health/Medication questionnaires, and amino-terminal proBNP (NT-proBNP), Hemoglobin A_{1c} and creatinine for calculating estimated Glomerular Filtration Rate (eGFR) surrogate biomarkers were excluded. **Results:** The LoB was -0.00092ng/L; LoD was 2.3 ng/L, which is 46% lower than the 5.0 ng/L LoD reported in TnI-II's FDA-submission. The total %CV was validated as <8% at 30 ng/L and 20 ng/L. After excluding 113(13.3%) USB-volunteers by surrogate biomarkers, the final healthy population had 734-members (See figure). 52.8% of females and 78.8% of males exceeded LoD. **Conclusions:** The cTnI-II system, FDA-cleared in 2011, fulfilled criteria as an hs-TnI assay. At the 99th-percentile CDVs, the total %CV for healthy female and male cohorts were ≤10%, and Greater than 50% of both healthy females and males exceeded the LoD.

Healthy Population Data after exclusion for eGFR<60 mL/min/1.73 m² OR HbA1c ≥ 6.5% OR NT-proBNP >125 ng/L if <75 years or >450 ng/L if ≥75 years.			
Description	**Overall**	**Females**	**Males**
Healthy populations, number of subjects	734	352	382
Number of cohort with TnI-II values > Limit of Detection (% of cohort > Limit of Detection)	487 (66.3%)	186 (52.8%)	301 (78.8%)
Non-parametric percentile method (CLSI C28-A3)			
99th percentile Clinical Decision Value	27.9 ng/L	20.3 ng/L	29.7 ng/L
90% Confidence Interval	90% CI: 20.1 – 29.7	90% CI: 12.8 – 29.7	90% CI: 21.2 – 36.9
Robust method (CLSI C28-A3)			
99th percentile Clinical Decision Value	14.0 ng/L	10.5 ng/L*	16.4 ng/L*
90% Confidence Interval	90% CI: 12.7 - 15.3	90% CI: 8.6 - 12.3	90% CI: 14.5 - 18.2
Harrell-Davis method			
99th percentile Clinical Decision Value	26.1 ng/L	21.0 ng/L	28.6 ng/L
90% Confidence Interval	90% CI: 20.7–31.5	90% CI: 13.9 - 28.0	90% CI: 23.9 – 33.3

*Differences for the 99th percentile cutoffs between the male and female cohorts achieved statistical significance.

A-082

Natriuretic Peptide NT-proBNP: Method comparison of two analysers.

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Background: Here we summarize the outcome of a comparison study to evaluate NT-proBNP assay. It has been performed at two analysers, AQT90 Flex from Radiometer®, a point of care technology, and cobas e801 from Roche Diagnostics®, the gold standard.Natriuretic peptides are secreted by the heart into the bloodstream as a result of an increase of intracardiac volumes and pressures. NT-proBNP has become an important biomarker of heart failure. The aim of the study is to compare the results and their interchangeability in order to determine the concordance between both immunoassays. **Methods:** The measurements were performed in serum samples from random real patients. The samples were processed in both analysers at the same day, in parallel. Statistical analysis was carried out with the MedCalc software, where the

correlation was calculated by the Pearson´s coefficient, the Passing-bablok regression and Bland Altman plots. **Results:** Below, there is a summary data table of the regression results.

Test	Instrument	Study Unit	N	Correlation coefficient Pearson r	Passing-Bablok		
NT-proBNP Serum	**x = AQT90 Flex; y = Cobas e801**	**pg/mL**	**124**	**0,9974** CI 95% = 0,9963 - 0,9982	**Slope= 0,93** CI 95% = 0,91 - 0,95**1 not included**	**Intercept= 0,79** CI 95% = (-5,52) - 5,40**0 included**	**Deviation from linearity P = 0,52 No significant deviation from linearity**

Results show a high degree of correlation coefficient and adjustment to linearity; however, there exists a **proportional bias**. It would be necessary to check the clinical concordance of the results, checking if this bias could be ignored under our working standard conditions.Concordance according to cut-off for heart failure was 96% (119/124). These five different results were in grey zone, very close to cut-off.**Conclusion:** Results from both analysers show a good correlation between the two methods. Due to the high clinical concordance, the proportional bias we found in the method comparison could be ignored and the interchangeability of methods is possible. Point of care technology offers a short response time what added to a good correlation results with the gold standard open an option to further accelerate the diagnosis of heart failure and thereby the initiation of adequate therapy.

A-083

Development and Evaluation of Analytical Performance of Immunoassay for the High Sensitive Measurement of Cardiac Troponin I for LUMIPULSE® L2400 Analyzer

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Background: Cardiac Troponin I (cTnI) and T (cTnT) are being used internationally as the standard biomarkers for the detection of myocardial injury, risk stratification in patients suspected of acute coronary syndrome (ACS) and for the diagnosis of myocardial infarction. In the recent international guidelines, algorithms are presented for rule-in and rule-out of non-ST-elevation myocardial infarction with the use of high-sensitivity (hs) cTnI or cTnT. We have developed new high sensitivity cTnI IVD kit, Lumipulse Presto hs Troponin I which is a fully automated chemiluminescence enzyme immunoassay (CLEIA) for LUMIPULSE L2400 analyzer. The higher throughput (240 tests/h) and STAT mode (approx. 15 minutes measurement time) of LUMIPULSE L2400 allows for quicker diagnosis. The analytical performance of the Lumipulse Presto hs Troponin I assay was evaluated, and compared with other hs cTn assays. **Methods:** Lumipulse Presto hs Troponin I is a two-step sandwich CLEIA. The resulting reaction signals are proportional to the amount of cTnI in the serum or plasma sample allowing quantitative determination of cTnI. Analytical performance of the assay was evaluated on LUMIPULSE L2400 analyzer (with STAT mode) according CLSI guidelines. **Results:** Limit of blank (LoB), detection (LoD) and quantitation (LoQ) were 0.5 pg/mL, 0.9 pg/mL and 2.7 pg/mL or less, respectively. The 99th percentile URL, imprecision at 99th percentile and detectable healthy population were estimated to be 21.0 pg/mL, 1.7%CV and 93%. Linearity was demonstrated over the range 2.5 to 46151.5 pg/mL. The coefficient of variation (CV) of total imprecision was 1.3 - 2.2%CV with 8 levels of samples. Results of method comparisons were correlation coefficient = 1.00, regression slope = 1.07 against Lumipulse G hs Troponin I, correlation coefficient = 1.00, regression slope = 0.98 against Architect high sensitivity Troponin I. While correlation with Roche hs Troponin T was correlation coefficient = 0.93, regression slope = 10.13. The measurement value variations by various interferences (bilirubin, hemoglobin, triglycerides, chyle, total protein, rheumatoid factor and HAMA) were ≤ 10% at the clinically high enough concentration. Unlike cTnT assay, no significant interference by hemoglobin was observed. **Conclusion:** Lumipulse Presto hs Troponin I assay met the latest criteria for "high-sensitivity" proposed by IFCC. The assay showed high assay precision, high robustness and high correlation with current hs-cTnI assays, Lumipulse G hs Troponin I and Architect high sensitivity Troponin I assays. It is expected that the new assay is useful as an aid in the diagnostics and risk management of ACS patients.

A-084

Human Epididymis protein 4 levels in acute cardiac failure

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Introduction:
Cardiac failure is a major health problem worldwide that concerns the health systems of developing countries. Cardiac failure is a clinical diagnosis based in specifics signs and symptoms but several laboratory markers had been proposed for its diagnosis. NT-proBNP is the only biomarker used for the diagnostic and prognostic of cardiac failure and it had been included in specific guidelines. There are a few studies that have seen association between HE4 (human epididymis protein 4)and acute heart failure severity in patients without tumoral pathology and normal renal function.
Objective:
We evaluated the level of human epididymis protein 4 (HE4)in selected patients with normal renal function, without gynecological pathology and non tumoral pathology described in the clinical histories
Methods:
22 patients that consulted the Emergency Department, with myocardial infarction were selected. Determination of NT-proBNP was made in the first 24 hours seeking for a laboratory diagnostic of hearth failure. The determination of NT-pro BNP and HE4 was made using an immunologic assay. To analyze the data we have used SPSS 16. Patients were divided in two groups using the NT-proBNP diagnostic value as recommended by the European Society of Cardiology with 88% positive predicting value: 1. Heart failure group (HFG). 13 patients (6 men and 7 women). 2. non Heart failure group (nHFG). 9 patients (4 men and 5 women) We calculated the medians and the IQR of both groups and the area under the curve (AUC), sensitivity and specificity were estimated
Results:
The mean age of HFG group was 62.22 and for the nHFG group was 70.25. No sex or age differences were observed. The HE4 median and IQR of HFG group and nHFG group was 185.44 (98.96) pmol/L and 59.50 (7.84) pmol/L respectively. We found statistically significant differences between both group (p=0.002). The AUC was 0.88 [IC95% = (0,71-1,00)] with 75% sensitivity and 100% specificity with a cutoff point of 97.1 pmol/L
Conclusions
The present study suggests a positive association between increased HE4 levels in acute cardiac failure. Further studies are needed to investigate the value of HE4 as a biomarker in acute hearth failure.

A-085

Analytical performance of the Elecsys® Troponin T Gen 5 STAT assay

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Background: The Elecsys® Troponin T Gen 5 Short Turn Around Time (TnT Gen 5 STAT; Roche Diagnostics) assay received FDA clearance in January 2017 and provides a high negative predictive value for ruling out acute myocardial infarction. We report analytical performance of this assay, including specificity versus diverse troponin isoforms. **Methods:** Precision was evaluated using human Li-heparin plasma and PreciControl Troponin samples per Clinical and Laboratory Standards Institute EP05-A2; two runs per day in duplicate for 21 days (n=84). Samples were measured using the Elecsys TnT Gen 5 STAT assay on the **cobas e** 411 and **cobas e** 601 analyzers (three reagents lots). Specificity for cardiac troponin T (at concentrations of 14ng/L, 4,000ng/L and 7,000ng/L) was tested versus skeletal muscle troponin T and I, cardiac troponin I, and human troponin C. Cross-reactivity with endogenous substances (including biotin) and commonly used/cardiac-specific drugs was tested at cardiac troponin T concentrations of 15ng/L and 9,000ng/L. Analytical specificity criteria were: recovery within ±1.4ng/L for cardiac troponin T concentrations <14ng/L; recovery within ±10% for cardiac troponin T concentrations ≥14ng/L. **Results:** On the **cobas e 601** analyzer, coefficient of variation (CV) ranges for repeatability and intermediate precision were 0.7-3.0% and 1.5-6.4%, respec-

tively (human Li-heparin plasma: 7.42-9,455ng/L; **Table**). On the **cobas e 411** analyzer, CV ranges for repeatability and intermediate precision were 0.7-5.6% and 1.4-10.3%, respectively. No interference was observed with skeletal muscle troponin T (up to 10,000ng/L) or I (up to 100,000ng/L), cardiac troponin I (up to 10,000ng/L), or human troponin C (up to 80,000ng/L). No interference was observed when biotin was tested up to 82nmol/L (20ng/mL) or with each of 16 commonly used/18 cardiac-specific drugs at the concentrations tested. **Conclusion:** The Elecsys TnT Gen 5 STAT assay demonstrated good analytical performance on **cobas e** 411 and **cobas e** 601 analyzers.

Intermediate precision and repeatability of the Elecsys TnT Gen 5 STAT on the Cobas e 601 analyzer

		Repeatability		Intermediate precision	
Sample	Mean, ng/L	SD, ng/L	CV, %	SD, ng/L	CV, %
PreciControl cTnT 1	24.2	0.27	1.1	0.77	3.2
PreciControl cTnT 2	1971	13.3	0.7	45.0	2.3
Human Plasma 1	7.42	0.22	3.0	0.47	6.4
Human Plasma 2	13.5	0.25	1.9	0.56	4.1
Human Plasma 3	154	1.23	0.8	2.24	1.5
Human Plasma 4	4831	38.0	0.8	124	2.6
Human Plasma 5	9455	62.7	0.7	256	2.7

Legend: SD, standard deviation; CV, coefficient of variation

A-086

Heart-type fatty acid-binding protein measurements to aid in interpreting abnormal and non-changing cardiac troponin concentrations

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Background: Stable or non-changing abnormal high-sensitivity cardiac troponin (hs-cTn) concentrations may indicate the presence of cardiac diseases other than acute coronary syndrome, or might even identify an analytical interference resulting in this high concentration. There are heterophile antibodies, autoimmune antibodies to cardiac troponin, and even macrocomplexes that may result in an abnormal hs-cTn concentration. When evaluating possible interferences affecting hs-cTn assays it may be useful to measure another cardiac biomarker using another type of methodology. In this regard, heart-type fatty acid binding protein (H-FABP), a biomarker that is released early after cardiac injury, and can be measured turbidimetrically on chemistry analyzers with open-channel capabilities might prove to be useful when investigating a possible analytical reason for abnormally high and non-changing hs-cTn concentrations. Our objective was to validate the Randox H-FABP on the Abbott ARCHITECT c8000 and to assess if measuring H-FABP in patients' samples with elevated and non-changing hs-cTn concentrations could identify analytical interferences. Methods: The Randox H-FABP assay, a latex particle-enhanced turbidimetric assay, was loaded on the Abbott ARICHTECT c8000 platform, with total imprecision at two concentrations assessed over 2 months, linearity evaluated (5 different concentrations), stability (freeze [- 80^{0}C]/thaw cycles and room temperature with EDTA plasma) assessed, with matrix comparison (lithium heparin versus EDTA plasma) and correlation with Abbott hs-cTnI concentrations in EDTA plasma. Also, EDTA plasma samples from patients with persistently elevated and stable cTnI concentrations (Abbott hs-cTnI≥52ng/L which equates to ≥2xULN/99th=26ng/L with change between results <20%) from patients with a primary discharge diagnosis not related to a cardiac etiology were collected and frozen (-20^{o}C). These samples were tested with the H-FABP assay (ULN/99th=6.3ng/L) and with polyethylene glycol (PEG) precipitation to identify macrocomplexes. Results: The imprecision (%CV) with Randox QC level 1 = 5.39 ug/L was 14.8% (n=40) and QC level 2 = 31.16 ug/L was 3.6% (n=37). The assay was linear from 3.8 ug/L to 95 ug/L. H-FABP was stable after 4 freeze/thaw cycles and, at room temperature, up to 150 hours in EDTA plasma as differences from baseline measurements (i.e., room temperature sample H-FABP = 12.50 ug/L and freeze/thaw sample H-FABP = 15.11 ug/L) were <20%. Comparison between lithium heparin and EDTA plasma samples for H-FABP was acceptable (mean bias=0.05ug/L, n=20 paired samples, with H-FABP range from 4.94 to 25.78 ug/L). The correlation between H-FABP and hs-cTnI results from 100 EDTA plasma samples was weak to moderate (Spearman's rho = 0.383 (95%CI: 0.201 to 0.539); p<0.001. During the validation, there were 4 patients with a non-cardiac discharge diagnosis with elevated and stable hs-cTnI concentrations ≥2xULN, all 4 had H-FABP concentrations <2xULN with 3 patients also having macrocomplexes that resulted in the high hs-cTnI concentration. Conclusion: The Randox H-FABP assay on the Abbott ARCHITECT c8000 analyzer yielded acceptable imprecision, linearity, and comparability between different matrices and under different storage conditions. H-FABP measurement might be useful when investigating patients with persistently high hs-cTn concentrations who do not have a clear cardiac etiology for this elevation; as the presence of macrocomplexes might be the cause for the elevation.

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Characterisation of microparticles in patients with acute coronary syndrome - a pilot study

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Background: Extracellular vesicles (EVs) in human blood can be subdivided into microparticles (MPs), 0.1-1µm in size, and exosomes below 0.1µm in size. MPs consist of a phospholipid bilayer and a substantial number expose procoagulant phosphatidylserine. They derive from different cell types upon proliferation, activation or apoptosis. It was shown that the number of MPs increases in thrombotic, inflammatory and hypoxic situations and it is suggested that they play an important pathophysiological role. We evaluated the amount and subtypes of MPs in patients with acute coronary syndrome (ACS) compared to a control group of patients presenting with chest pain, but without ACS. The characterization of MPs in ACS may give a better insight into the pathophysiology of the disease and add prognostic relevant information for the risk stratification. **Methods:** This is an ongoing study that recruited patients with thoracic pain suggestive for ACS from the emergency unit of the Kantonsspital Aarau/Switzerland and from the University Hospital in Vienna/Austria. Patients with recent myocardial infarction, malignancy, pulmonal embolism, pneumonia, sepsis or acute infection, severe heart or renal failure were excluded as in these patients increased MPs are suspected. Preliminary results derive from the first center, where patients have been divided into ACS-positive (n=26) and ACS-negative (n=36) groups are presented here. Citrate blood was immediately drawn upon presentation (t1), and for ACS-patients also approx. 4 hours later (t2) and on the next day (t3). Blood tubes were transported to the laboratory on foot without agitation and immediately centrifuged two times to receive platelet free plasma (PFP). PFP was stored at -80°C until batch analysis using a 4 laser flow cytometer (CytoFLEX, Beckman Coulter). The gate for MPs was set with silica beads and triggering was done on Annexin-V Cy5. Different MPs were investigated using calcein and Annexin-V combined with fluorescence labeled antibodies against erythrocyte (EMPs), platelet (PMPs), monocyte (MMPs) and endothelium (EnMPs) derived MPs. Additionally, CRP and N-terminal pro B-type natriuretic peptide (NT-proBNP; both on a Dimension Vista from Siemens Healthineers) and high-sensitivity cardiac troponin I (hs-cTnI; Architect analyser from Abbott Diagnostics) were measured. Mann-Whitney U test for between group comparison, Wilcoxon test for within group comparison and Spearman rank correlations were performed by SPSS 24. **Results:** These preliminary results show that Annexin-V positiv MP levels were significantly increased in ACS-patients at t2 and t3 compared to controls (p=0.008 and p=0.038, respectively) and between ACS patients at t1 and t2 (p=0.039). EnMP concentrations (CD31+CD54+CD146+CD42-) were significantly higher in ACS-patients than in controls upon presentation (p=0.03) and significantly higher at t2 in ACS compared to controls (p=0.016). Similar results were found for the subgroup of NSTEMI patients. There was a significant correlation between EnMPs and CRP (r=0.388, p=0.002), hs-cTnI (r=0.408, p=0.002) and NT-proBNP (r=0.486, p<0.001). **Conclusion:** Annexin-V positive MPs and EnMPs were significantly increased in ACS patients compared to controls. Further, EnMP concentrations correlated significantly with established cardiac markers, suggesting Annexin-V and EnMP as prospec-

tive candidates for the evaluation of their prognostic value in a follow-up of ACS patients.

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Performance Evaluation of Atellica IM High-Sensitivity Troponin I Assay in a CORE Laboratory

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Background: The objective of this study was to verify the analytical performance (precision) of the Siemens Healthineers Atellica IM High-Sensitivity Troponin I (TnIH) Assay on the Atellica® IM Analyzer, and perform method comparison vs. ADVIA Centaur® High-Sensitivity Troponin I (TNIH) assay, and Dimension EXL® High Sensitivity Troponin I (TNIH) assay. In addition, the effects of hemolysis and biotin were assessed. **Methods**: The assay is a dual-capture sandwich immunoassay using magnetic latex particles, a proprietary acridinium ester for chemiluminescence detection, and three monoclonal antibodies. Precision studies were performed according to CLSI protocols EP05-A3 and EP15-A3 using lithium heparin plasma samples - two sample pools (SP), and three levels of controls. One aliquot of each sample pool and each QC material was tested in replicate in two runs per day on each analyzer for a minimum of ten days with one lot of reagent and calibrator. Each run was separated by at least a two hour time interval. A total minimum of 40 replicates were generated per sample. Hemolyzed samples (150 mg/dL and 500 mg/dL) and samples spiked with biotin (30 and 1500 ng/mL) were run in duplicate on the Atellica IM TnIH Assay. **Results:** Precision studies agreed with the manufacturer's claims: Within day (repeatability) CV%(SD)s were 3.1(0.36), 2.7(0.69), 1.7(1.66), 2.2(5.60), and 1.5(84.62) for concentrations of 11.43(SP), 25.57(SP), 98.48, 259.22, and 5805.88 ng/L; within lab (total) CV%(SD)s were 5.8(0.66), 4.5(1.15), 2.6(2.60), 2.8(7.20), and 1.5(85.29) respectively. Method comparison of Atellica IM TnIH Assay vs. ADVIA Centaur TNIH assay showed a regression slope of 0.88 (95%CI 0.862 to 0.902), intercept of 0.77 (95%CI 0.33 to 2.61), and correlation coefficient r=0.997 (n=39); and, Atellica IM TnIH Assay vs. Dimension EXL TNIH assay showed a regression slope of 0.97 (95%CI 0.933 to 1.01), intercept of -0.24 (95%CI -6.13 to 9.48) and correlation coefficient r=0.998 (n=0.998). All hemolysis (up to 500 mg/dL) and biotin (up to 1500 ng/mL) samples tested with the Atellica IM TnIH Assay demonstrated ≤10% change in results. **Conclusion:** The Atellica IM TnIH Assay has demonstrated good precision for detecting low cardiac troponin I concentrations and good correlation with the Dimension EXL TNIH assay and a slight negative bias with the ADVIA Centaur TNIH Assay. At levels of biotin up to 1500 ng/mL and hemolysis up to 500 mg/dL, there was ≤10% change in results.

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Atellica IM High-Sensitivity Troponin I Assay: Analytical Evaluation Among University Hospitals

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Siemens Healthineers supported the study by providing systems, reagents, and protocols, and contributed to data analysis. **Background:** Cardiac troponin is the favorite biomarker for aiding in the diagnosis of myocardial infarction (MI). By definition, high sensitivity troponin assays must demonstrate increased analytical sensitivities and gain of precision at the lower concentrations, allowing the time point shortening of serial measurements, and improving their diagnostic sensitivity for early MI detection. The Atellica® IM High-Sensitivity Troponin I (TnIH) Assay is an *in vitro* diagnostic immunoassay for the quantitative determination of cardiac troponin I (cTnI) in serum or plasma (lithium heparin). The goal of this study was to check TnIH Assay analytical precision run on Atellica® IM 1600 Analyzer, and to compare TnIH Assay to Abbott ARCHITECT STAT High Sensitive Troponin-I (ARCHITECT TnIhs) and ADVIA Centaur High-Sensitivity Troponin I (TNIH) assays. **Methods:** The Atellica IM TnIH Assay is a dual-capture sandwich immunoassay using three monoclonal antibodies, magnetic latex particles and an unique proprietary acridinium ester for chemiluminescence detection. Precision studies were performed according to CLSI protocols EP05-A3 and EP15-A2 using lithium heparin plasma samples (two sample pools), and three levels of quality controls (QC). One aliquot from each pool and each QC material was tested in duplicate using one lot of reagent and calibrator (two runs per day on each analyzer) during at least ten days. Each run was kept apart by at least a two hour time interval. At least 40 replicates were generated for each of the two sample pools and the three levels of QC. Method comparison was performed according to EP09-A3. cTnI samples (lithium heparin) were obtained from acute chest pain patients selected in three university Emergency Departments (Bichat, Beaujon and Tenon). Serial samples were collected on two times, one at admission and one within 1, 2, 3, or up to 6 h later and were tested using the three cTnI assays. Assay comparisons were made using Deming correlation. **Results:** Within run repeatability for cTnI concentrations of 10.7, 25.0, 95.0, 252.8, 5717.0 ng/L (CV%(SD) were 3.6 (0.38), 2.9 (0.73), 1.6 (1.50), 1.7 (4.35), and 1.2 (67.03). Within lab (total) CV%(SD)s were 6.8 (0.72), 3.4 (0.85), 4.0 (3.78), 4.3 (10.93), 2.7 (154.90), respectively. Atellica IM TnIH Assay comparison with Abbott ARCHITECT TnIhs assay (range 0.28 ng/L to 15,989 ng/L for Atellica IM TnIH Assay and range 0.2 ng to 30,602 ng/L for Abbott ARCHITECT assay; n=99) showed a slope of 1.01 (95%CI 0.885 to 1.152) and intercept of 0.77 (95%CI -0.195 to 1.898), r=0.964, and with ADVIA Centaur TNIH assay (range 0.28 ng to15,988 ng/L for Atellica IM TnIH assay and range 0.36 ng to 16,473 ng/L for ADVIA Centaur TNIH assay; n=97) a slope of 1.02 (95%CI 1.004 to 1.039) and intercept of 0.68 (95%CI 0.437 to 1.065), r=0.999. **Conclusion:** The Atellica IM TnIH Assay demonstrated acceptable precision for detecting low cTnI concentrations and confirmed the manufacturer's claims. Furthermore, no significant analytical bias was found when compared to two commercially available high sensitivity cTnI assays.

A-090

Will different clinical cut-offs impact the diagnostic accuracy of hs-cTnI assays in suspected ACS patients?

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Background: BACKGROUND: Chest pain is a common cause of hospital admission world widely and is a major burden on healthcare resources. Cardiac troponin assays have substantially improved the accuracy of diagnosis and prognostic assessment of patients with suspected acute coronary syndrome (ACS). We aim to investigate the influence of different choices of cut-offs for high sensitivity cardiac troponin I (hs-cTnI) assays in patient admission or discharge (according to assigned color code and pain during triage), in order to identify the best senario in terms of diagnostic accuracy and the need of hospitalization. **Methods:** METHODS: A retrospective analysis was conducted on 586 Emergency Department (ED) patient records within a month who had chest pain complaints in 2017. Only patients who were diagnosed with ACS were included in the analysis. Patients with non-cardiac diagnosis such as thoracic trauma were excluded. All eligible patient samples were measured using Beckman Coulter Access hsTnI and Abbott ARCHITECT hsTnI assays. We investigated three different scenarios using established hs-cTnI cut-off values for ruling in chest pain patients in our population: Beckman Coulter Access hsTnI's manufacture insert (male 19.8 ng/L and female 11.6 ng/L), gender specific 99th percentile cut-offs from Abbott hsTnI's manufacture insert (male 34 ng/L and female 15 ng/L) and 12 ng/L recommended by Dr. Shah's group for Abbott hsTnI (NCT01852123). Factors that could impact the need of hospitalization were further analyzed. **Results:** RESULTS: We included 338 patients (178 men and 160 women) with ACS diagnosis in our final analysis. The need of hospitalization was associated with troponin values above the hs-cTnI cut-offs adopted in each scenario with statistical significance (Abbott, p-value < 0.001; Beckman Coulter, p-value < 0.001; Shah, p-value < 0.001). No statistically significant difference was found among the three scenarios using various hs-cTnI cut-offs in identifying hospitalized patients. Moreover, the higher hsTnI cut-off is associated with an increased probability of admission, corrected for age, gender and color code (Abbott odds ratio (OR) 7.74, 95% CI 2.89-20.75, p<0.001; Beckman 3.93, 95%CI 1.89-8.18, p < 0.001; Shah 5.06, 95% CI 2.51-10.22, p < 0.001). The hospitalization is highly associated with the color code (p<0.001) given during the triage. **Conclusion:** CONCLUSION: In our patient population, there is no statistically significant difference among the three scenarios adopting different hs-cTnI cut-offs in identifying hospitalized patients. There is a statistically significant association observed between the color code given during the triage, the hs-cTnI level and the hospitalization. Therefore, the appropriate use of hs-cTnI assays is the key to the correct diagnosis.

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High-sensitivity cardiac troponin T assay has increased susceptibility to biotin interference

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Background: Biotin, vitamin B7, can interfere in assays that have streptavidin-biotin interaction as a part of their assay reaction. The high-sensitivity cardiac troponin T generation 5 assay (hs-cTnT) now available in the US is one of the assays vulnerable to biotin interference because of its assay design. Given the importance of the hs-cTnT assay in rapidly ruling-in and ruling-out acute myocardial infarction (AMI) in patients presenting with chest pain in the emergency department (ED)[2], we sought to evaluate the extent of biotin interference in the new assay in comparison to the contemporary 4th generation troponin T assay (cTnT). Further we sought to estimate the impact of biotin interference in hs-cTnT in ruling out AMI through simulations based pharmacokinetic studies. **Methods:** This was a University hospital, laboratory based study of discarded blood samples received from patients presenting to the ED and referred for troponin measurement. 23 cTnT-positive patient samples were tested by hs-cTnT and cTnT assays after adding known plasma concentrations of biotin achievable from a 5-day course of biotin, 10 mg daily. Maximum plasma biotin concentrations of 140, 100 and 50 ng/ml achievable at 1, 2 and 4 h after the last dose taken on the 5th day were simulated. Next, biotin spiking experiment with a wide range of plasma biotin concentrations (10-2000 ng/ml), as required by the FDA was undertaken in cTnT positive patient samples. In the presence of biotin, false decreases >10% or suppression of cTnT values below the 99th percentile of upper reference limit and/or our institution's threshold for abnormal (hs-cTnT≥52 ng/L; cTnT≥0.01 ng/ml) were considered significant. **Results:** A simulation of daily biotin use in 23 cTnT-positive patient samples resulted in significant interference in hs-cTnT values compared with the cTnT assay. 78% and 33% of hs-cTnT results were falsely decreased below the upper reference limit of 19 ng/L at 140 and 100 ng/ml concentration of plasma biotin, respectively. Among 12 samples that were significantly abnormal (hs-cTnT≥52 ng/L), 83%, 70%, and 29% had values <52 ng/L at 1, 2, and 4 h post-dose biotin simulations, respectively. In contrast, cTnT results remained unaffected at these plasma biotin concentrations. In dose-dependent biotin testing, the hs-cTnT assay was susceptible to biotin interference at plasma biotin >31 ng/ml compared with a threshold >315 ng/ml for the cTnT assay. **Conclusions:** Our data suggest a significant risk of false rule-out or delayed rule-in of AMI in the presence of biotin with hs-cTnT, far more than with the prior cTnT assay, at plasma biotin concentrations reflecting those contained in commonly used over-the-counter supplements. We suggest careful history taking for OTC supplements in patients presenting for rule out of AMI using the hs-cTnT assay. We also suggest the manufacturer work to increase the biotin tolerance levels of the assay or convert it to a non-biotinylated assay. Finally, we advise health care systems using this assay to make ordering providers aware of the potential for biotin interference in hs-cTnT levels.

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Performance Evaluation of the VITROS® hs Troponin I Assay* on the VITROS® 5600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems

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Background: The Joint European Society of Cardiology/American College of Cardiology guidelines state that cardiac troponins are the preferred biomarkers for the detection of myocardial injury, for risk stratification in patients diagnosed with acute coronary syndrome, and for the diagnosis of myocardial infarction. Because of the demand for accurate and precise measurement of low troponin levels, there is an increased need for assays with improved analytical performance. **Methods:** We are developing a rapid, fully automated high sensitivity assay for the measurement of cardiac Troponin I (cTnI) in human serum and plasma (heparin) for use on the VITROS® Systems. The VITROS® hs Troponin I (hsTnI) assay uses an immunometric technique in which the cTnI present in the sample reacts simultaneously with one streptavidin-conjugated antibody, bound by biotin-BSA on the wells, and a dual antibody-horseradish peroxidase conjugate. The antigen-antibody complex is captured by the antibody coated on the wells. Unbound materials are removed by washing, and the bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of cTnI present in the sample. The time to first result in the system is 15 minutes. **Results:** Preliminary data show an assay range of 1.70 - 30,000 ng/L. The Limit of Blank, Limit of Detection, and Limit of Quantitation (LoQ) of 0.29 ng/L, 1.04 ng/L, and 1.70ng/L (20% CV) respectively were established according to CLSI-EP-17-A2. The LoQ concentration at 10%CV was 4.34 ng/L. In a CLSI-EP05-A3 precision study, five precision pools with mean cTnI concentrations of 2.19, 21.45, 131.0, 523.1 and 17,778 ng/L had within-run percent coefficient of variation (%CV) of 12.71%, 1.53%, 1.45%, 1.26%, and 1.67% respectively and within-laboratory %CV of 19.33%, 5.54%, 3.16%, 3.46%, and 3.42% respectively. Correlation between the VITROS hsTnI assay and the contemporary VITROS Troponin I ES (TropIES) assay was obtained using 123 patient samples spanning the common measuring range of the two assays. The regression statistics, using Passing and Bablock, were as follows: VITROS hsTnI = 1.02*TropIES - 4.51; Pearson Correlation Coefficient (r) = 1.00. **Conclusion:** In summary, the VITROS® hs Troponin I assay demonstrates reliable and acceptable performance on the VITROS® 5600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems. *Under Development

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Human cardiac TnI degradation and antibody selection for the assay development

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Background: The measurement of cardiac troponin I (cTnI) in blood is one of the most trusted methods of acute myocardial infarction (AMI) diagnosis. However, in spite of a long history of this biomarker in clinical practice, the selection of antibodies for new generations of cTnI assays remains a complex task. Over the last decade it was shown that the samples of some patients contain autoantibodies that negatively interfere with most of the immunoassay mAbs which are specific to the central (~40-130 amino acid residues, aar) fragment of cTnI (which is considered to be the most stable part of the cTnI molecule). In the current study we aimed: a) to analyze the dynamics of cTnI degradation after AMI, and b) to border cTnI proteolytic fragments that are presented in the circulation of AMI patients in order to determine the epitopes of antibodies that are not significantly influenced by the proteolytic degradation. **Methods:** Serial blood samples were collected from 66 patients over a period of 1-36 hours following the onset of AMI, both before and after stenting. cTnI and its fragments were studied by Western blotting and fluoroimmunoassay analysis. **Results:** In the blood of all AMI patients, cTnI was presented by an intact molecule and 11 major fragments with relative molecular masses of 14-24 kDa. Stenting neither affected the repertoire nor the ratio of different cTnI fragments. The ratio of full sized cTnI and its fragments did not change considerably within the first 36 hours after the onset of AMI. mAbs with the epitopes located between ~23-196 aar recognized more than 80% of all detected cTnI. **Conclusion:** The composition of cTnI fragments in the circulation is mainly constant within the first 36 hours following AMI. More than 80% of all detected fragments comprise 23-196 aar of cTnI which enables the utilization in immunoassays antibodies that are specific to the regions 23-40 and/or 140-196 that are only mildly affected by autoantibody interference.

A-094

Serum Gamma-Glutamyltransferase Levels are Associated with Cardiovascular Risk Factors in China:A Nationwide Population-Based Study

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Background: Serum Gamma-Glutamyltransferase (GGT), which is mainly derived from the liver, is a sensitive marker of liver cell damage and oxidative stress. More recently, it has been found that the increased plasma activity of GGT is also associated with cardiovascular disease (CVD). However, data on the relationship between GGT and cardiovascular risk factors (CRFs) are lacking in nationally representative samples of the Chinese population. Here, we aim to investigate both the association between GGT and CRFs and CRFs clustering. Methods: A cross-sectional survey was conducted in a nationally representative sample of 22897 adults aged 18 years and older from 2007 to 2011, including a plurality of ethnic minorities. Questionnaires and physical examinations were performed, and laboratory measurements were collected. The participants were then divided into quartiles of sex-specific serum GGT. Results: People in Northern and Rural areas tended to have a greater chance of belonging to the upper quartiles of GGT, and for ethnic groups, Mongolians had the highest serum level of GGT. From the low to the high GGT quartile, the incidence of each CRF and clustered risk factors increased after adjusting for age, uric acid (UA), drinking, ethnicity and all other risk factors. Subjects in the upper stratum (>75th percentile) had higher prevalence rates of CRFs than did those in the lower stratum. Furthermore, the individuals with clustering of 1, 2 or ≥3 CRFs were still more likely to belong to the upper GGT quartiles (75th percentiles) than were those without risk factors in both genders. Conclusion: Our data highlight the association between higher serum GGT levels and CRFs in Chinese adults. We found that people with higher serum GGT levels tend to have a greater chance of CRFs and that there was a dose-response association between the number of CRFs and higher serum GGT, especially in men, suggesting that serum GGT may serve as a valuable clinical marker of cardiovascular disease in China. Further studies are needed to elucidate the causality between serum GGT and CRFs and to evaluate the effects of serum GGT lowering therapies on CVD prevention and outcome.

A-095

Sex-specific versus universal clinical decision limits for troponin I and T for the diagnosis of acute myocardial infarction -a systematic review

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Background: The universal clinical decision limits of high-sensitivity cardiac troponin I (hs-cTnI, 26 ng/L) and T (hs-cTnT, 14 ng/L) may contribute to underdiagnosis of acute myocardial infarction in women. We performed a systematic review to investigate sex-specific and universal 99th percentiles of hs-cTnI and hs-cTnT derived from healthy reference populations. **Methods:** We searched in PubMed and EMBASE for original studies, and by screening reference lists. Reference populations designed to establish 99th percentiles of hs-cTnI(Abbott) and/or hs-cTnT(Roche), published between January 2009 and October 2017, were included. Sex-specific and universal 99th percentile values of hs-cTnI and hs-cTnT were compared with universal clinical decision ranges (hs-cTnI: 23.3-29.7 ng/L, hs-cTnT: 12.7-24.9 ng/L). **Results:** A total of 28 studies were included in the systematic review. Of 16 hs-cTnI and 18 hs-cTnT studies, 14 (87.5%) and 11 (61.1%) studies reported lower female-specific hs-cTn cut-offs than universal clinical decision ranges, respectively. Contrary, men-specific thresholds of both hs-cTnI and hs-cTnT were in line with currently used universal thresholds, particularly hs-cTnT (90% concordance). The variation of estimated universal 99th percentiles was much higher for hs-cTnI than hs-cTnT (29.4% versus 80.0% of hs-cTnI and hs-cTnT studies reported values within the current universal clinical decision range, respectively). **Conclusion:** Our data show substantially lower female-specific upper reference limits of hs-cTnI and hs-cTnT than universal clinical decision limits of 26 ng/L and 14 ng/L, respectively. The statistical approach strongly affects for the hs-cTnI threshold. Downwards adjustment of hs-cTn thresholds in women may be warranted, to reduce underdiagnosis of acute myocardial infarction in women.

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Decision limits, delta troponin or both for the confirmation and exclusion of myocardial infarction using contemporary and high sensitive assays

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Objective: To examine the impact of a combination of a delta troponin with different decision limits for the rapid confirmation or exclusion of myocardial infarction (MI). 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 30-50,000 ng/L 10% CV 60ng/L 99th percentile 70 ng/L; the Beckman AccuTnI enhanced (B) (Access 2 , Beckman-Coulter) range 1 - 100,000 ng/L, 10% CV 30 ng/L, 99th percentile 40 ng/L, the Siemens Ultra (S) (ADVIA Centaur, Siemens Healthcare Diagnostics), range 6 - 50,000 ng/L, 10% CV 30 ng/L 99th percentile 50 ng/L. and cardiac troponin T (cTnT) by the Roche high sensitivity cardiac troponin T assay hs-cTnT (Elecsys 2010, Roche diagnostics), range 3 - 10,000ng/L, 10% CV 13ng/L, 99th percentile 14 ng/L. The universal definition of myocardial infarction utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. Myocardial infarction was diagnosed by a value exceeding the 99th percentile and/or the combination of a delta troponin. Myocardial infarction was excluded when either the admission or all values fell below the limit of detection of the assay and there was no delta troponin. All other patients were classed as non-diagnostic. Results: Samples were available from 813 and serial samples in 617 /1132 patients enrolled in the study, 60% male, age 23.7-92.8 years median 53.8 years. The admission sample below the limit of detection (LOD) missed 0.6-1.2% of patients. Both samples remaining below the LOD of the assay with no delta change excluded myocardial infarction in 99.8% of cases and was associated with a MACE rate of 0.2-0.3%, all of which were readmissions with acute coronary syndrome. Use of a delta change did not improve detection of MI but increased the number of false positive diagnoses by 0.1-1.5%. Conclusion: Serial measurements are required for reliable rule out of MI. Troponin below the limit of detection measured with a sensitive or contemporary sensitive assay without a delta change identified a very low risk group who can be considered for immediate further investigation or discharge. The 99th percentile alone on serial sampling was the most effective. Rule in with a delta in addition generated false positive results.

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Study of the association between bone mineral disorders <and> intradialytic hypertension in patients on maintenance hemodialysis

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Background:
Intradialytic hypertension (ID-HTN) affects up to 15% of hemodialysis (HD) patients and is associated with significant risk of hospitalization and death. Abdominal aortic calcification affects 81% of MHD patients with its severity increases with age, duration of dialysis and history of cardiovascular disease (CVD). Mineral Bone Disease (CKD-MBD) related factors such as serum calcium, phosphorus, and parathyroid hormone are strong-

ly associated with severity of Aortic calcification (AC) in HD patients. FGF-23 level increases progressively in CKD patients, beginning in its early stages achieving the highest values in end-stage renal disease (ESRD) patients, to maintain normal serum phosphate levels. Elevated FGF-23 has been linked with hypertension, left ventricular hypertrophy and increased cardiac mortality in CKD patients, but its role in pathogenesis of ID-HTN via inducing vascular calcification and stiffness remains to be explored.
Methods:
This study included sixty ESRD patients on regular HD for more than 6 months, that were classified into two groups; Group *(1):* forty five ID-HTN prone HD patients who developed episodes of ID-HTN in more than 2/3 of HD sessions done during last 3 consecutive weeks, and *Group (2):* fifteen hemodynamically stable (S) HD patients, without history of ID-HTN as a control group. To all subjects, laboratory investigations were performed including pre- dialysis serum urea, creatinine, electrolytes, minerals, iPTH and FGF-23. Abdominal aortic calcification score (AACS) was assessed in lateral abdominal radiographs by Kauppila method. Atherosclerosis score (AS) was calculated based on measurement of carotid intima media thickness (CIMT), detection of carotid plaques with or without significant stenosis and measurement of ankle brachial BP index.
Results:
Hypertension prone (HP) patients had significantly longer duration of dialysis and higher AACS compared with hemodynamically stable (S) patients. Serum phosphorus, calcium phosphorus product (CaPhP), iPTH and FGF23 were higher in HP than S patients, but the difference was not statistically significant. There was a statistically significant positive correlation between FGF23 and each of duration of dialysis (P = 0.003) and CIMT (P = 0.043). Moreover, AS had a statistically significant positive correlation with serum calcium (P = 0.009).
Conclusion:
The occurrence of ID-HTN is associated with significantly more advanced vascular calcification and fairly increased levels of humoral MBD mediators involved in this process like FGF23, iPTH and CaPhP. FGF23 significantly correlates with CIMT, possibly indicating its involvement in the atherosclerotic process from the early beginning. It remains to be elucidated whether interventions to control FGF23 rise and other MBD parameters would reduce ID-HTN episodes.

A-098

Analytical Evaluation of a New Ultra-Sensitivity Troponin I Assay using Human Serum

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Background: The universal definition of acute myocardial infarction (AMI) puts cardiac troponin at the forefront of diagnosis. With the advent of high-sensitivity assays for cardiac troponins the diagnosis of AMI can be made sooner with a higher degree of confidence. The objective of this study was to provide an analytical evaluation of a newly developed ultra-sensitivity cardiac troponin I assay (us-cTnI), using serum samples.
Methods: Us-cTnI was measured using the Sgx Clarity™ cTnI Assay (us-$TnI_{(Singulex)}$) on the Singulex Clarity System, with a reported limit of detection (LoD) of 0.08 ng/L and a 99th percentile upper reference limit (URL) of 8.67 ng/L in EDTA plasma. The us-$TnI_{(Singulex)}$ limit of blank (LoB) and LoD were calculated in this study after measuring the zero calibrator 22 times. Imprecision profile, linearity and sample stability were determined using pooled human serum samples. A sample type comparison was performed with paired serum and EDTA plasma. The 99th percentile URL was calculated with serum from 638 apparently healthy individuals (318 females and 320 males) using the Harrell-Davis quantile bootstrap statistical method. Receiver operating characteristic (ROC) curves were used to compare the clinical performance of the us-$TnI_{(Singulex)}$ with the hs-$cTnI_{(Abbott)}$ and hs-$cTnT_{(Roche)}$ assays in a limited study using serum; the hs-$cTnI_{(Abbott)}$ and hs-$cTnT_{(Roche)}$ 99th percentile URLs of 26.2 ng/L and 14 ng/L, respectively, were used as the diagnostic cut-offs. The hs-$cTnI_{(Abbott)}$ assay has a LoD of 1.1-1.9 ng/L and a 10% CV of 4.7 ng/L; the hs-$cTnT_{(Roche)}$ has a LoD of 5 ng/L and a 10% CV of 13 ng/L.
Results: The LoB was 0.01 ng/L and the LoD 0.04 ng/L. The 10% CV from the imprecision profile was 0.49 ng/L and the assay was linear from 0.5-20,000 ng/L. Serum samples with a cTnI concentration ranging from 0.59-18,148 ng/L had an overall positive bias of 36% when compared to EDTA plasma. Serum samples were stable for five days and for five freeze-thaw cycles at -20^{o}C, four days at 4^{o}C and for two days at room temperature. The overall 99th percentile URL was 4.33 ng/L (95% CI 2.83-5.72 ng/L) with 99.8% of individuals having a cTnI concentration above the LoD. The female 99th percentile URL was 2.53 ng/L (95% CI 1.66-3.99 ng/L) and the male 5.53 ng/L (95% CI 3.73-7.25 ng/L). Area under the ROC curves for the us-$TnI_{(Singulex)}$ when compared to the hs-$cTnI_{(Abbott)}$ and hs-$cTnT_{(Roche)}$ assays were 0.900 (95% CI 0.805-0.995) and 0.927 (95% CI 0.860-0.993), respectively.
Conclusion: The us-$TnI_{(Singulex)}$ assay had a LoB, LoD and 10% CV lower than any other assay currently on the market, making it the most sensitive cardiac troponin assay available. Sample stability was acceptable enough to allow both clinical and research applications. There was a matrix effect in serum when compared to EDTA plasma. The 99th percentile URL in serum was lower than that reported in EDTA plasma, which likely reflects differences in the underlying reference populations. There was an expected male/female difference in serum 99th percentile URL. Clinical diagnostic performance of the us-$TnI_{(Singulex)}$ assay appeared better than the predicate assays tested.

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Simultaneous Assessment of N-terminal pro-B-type Natriuretic Peptide and Presepsin Improves Risk Prediction of Acute Kidney Injury and Mortality after Cardiac Surgery

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Background
Acute kidney injury (AKI) is common after cardiac surgery. Also sepsis has shown to contribute to the development of AKI in intensive care patients. Presepsin (PSEP) has proven as a marker with high diagnostic and prognostic validity in assessment of disease severity and association to kidney function in septic patients. N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels reflect cardiac filling pressures. Therefore NT-proBNP is a surrogate marker for hemodynamic status. It has been shown association of NT-proBNP with AKI and cardiac events after cardiac surgery.
Objective
The aim of the present study was to evaluate the diagnostic validity of NT-proBNP and PSEP to predict the risk of cardiac surgery-associated AKI (CSA-AKI) and postoperative mortality in comparison with the inflammatory markers C-reactive Protein (CRP) and procalcitonin (PCT) and creatinine.
Methods
The marker concentrations were measured in plasma samples which were drawn in the early morning after surgery from 856 patients undergoing elective cardiac surgery. Outcome measures were postsurgical AKI during hospitalization and mortality. PSEP and NT-proBNP were determined by using PATHFAST Presepsin (LSI Medience corporation, Tokyo) and Elecsys NT-proBNP (Roche Diagnostics). CRP, PCT and creatinine were measured using routine clinical chemistry methods in the central laboratory.
Results
Patients who developed AKI (n=221, *25.8%*) had higher PSEP and NT-proBNP levels than patients without AKI (PSEP: 632 ng/L (IQR 378-1069) *versus* 304 ng/L (228-434), Difference 328 ng/L, p<0.0001; NT-proBNP: 1594 ng/L (IQR 667-3838) *versus* 352 ng/L (IQR 122-1084), Difference 1242 ng/L, p<0.0001. The results for 6-month death (n=49, *5.8%*) were PSEP: 337 ng/L (IQR 246-512) *versus* 1081 (511-1962), Difference 744 ng/L, p<0.0001 and NT-proBNP: 499 ng/L (IQR 161-1475) *versus* 2632 ng/L (IQR 1308-3874), Difference 2133 ng/L, p<0.0001 for survivors *versus* non-survivors, respectively. AKI has been assessed according to AKIN classification: stages 1 (n=122), 2 (n=54), 3 (n=45). The marker concentrations increased significantly from AKIN 1 to 3. Receiver operator curve (ROC) analysis for prediction of 6-month death revealed AUC values of 0.792 and 0.847 for NT-proBNP and PSEP compared to AUC values of 0.670, 0.778 and 0.639 for creatinine, PCT and CRP, respectively. Similar results were obtained for prediction of postsurgical AKI by ROC analysis. AUC values were 0.758 and 0.783 for NT-proBNP and PSEP, compared to AUC values of 0.671, 0.671 and 0.512 for creatinine, PCT and CRP, respectively. Examination of the predictive value of marker combinations by logistic regression revealed an AUC value of 0.796 for the combination PSEP and NT-proBNP. This finding demonstrated superiority of the simultaneous assessment by using the combination NT-proBNP and PSEP for the risk of developing postsurgical AKI compared to the markers alone and to all other possible marker combinations.
Conclusion
PSEP and NT-proBNP demonstrated comparable predictive power for risk of 6-month mortality after cardiac surgery and to identify patients who were at risk of developing CSA-AKI. Moreover, the combination of both markers was found to improve the prognostic performance. The simultaneous assessment of NT-proBNP and PSEP allows early risk prediction of AKI already at the first day after surgery and may enable individual risk stratification with appropriate individualized patient care.

A-100

The implementation of the high sensitivity Troponin T (hs-TnT) generation five assay at a large teaching county hospital. A multi-speciality effort

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Background
A highly sensitive Troponin T assay (hs-TnT) was recently approved by the FDA for clinical use. In addition to the assay's much improved sensitivity, the reporting units, reference intervals and critical limits for notification are markedly different. Furthermore, current acute myocardial infarction (AMI) rule out protocol requires serial samples and monitoring for up to 6 hours and it is not known if adopting hs-TnT will impact the protocol. This report describes the implementation of the hs-TnT assay at a large teaching county hospital.
Methods:
A multi-speciality team from Clinical Chemistry, Laboratory Administration and Information Technology support services, Nursing, Cardiology, Hospitalists, Performance Improvement and Emergency departments was set up. The hs-TnT assay was validated as per protocol on the Cobas® 6000 system, e601 immunoanalyzer (Roche Diagnostics) for analytical performance. Samples received into the laboratory from patients being investigated for acute coronary syndrome were analysed using the conventional 4th generation TnT assay and results reported in the usual manner. The Fifth generation hs-TnT was also measured but results not reported to the electronic medical record. The imprecision of the assay was assessed at recommended decision levels. A new test code was set up in the Laboratory Information System, for the hs-TnT with test specific parameters for measurements limits and critical value. At three months of data and clinical correlation, educational materials were prepared and training sessions conducted for pathology residents and staff, nursing staff, and medical staff, by the clinical chemist, nursing education, and cardiologists respectively.
Results:
969 samples from 541 patients (56% men, 44% women) being investigated for acute coronary syndrome were analysed for both conventional and fifth generation hs-TnT. There was no numerical correlation between the two assays on admission (P=0.39). Laboratory results of both assays correlated well when using clinical assessment of patients. A two step one hour and 3 hours rule-out protocol was developed. The imprecision of the hs-TnT assay ranged from 0% at 12.0 ng/L, 3.3 % at 16.4 ng/L, 2.2% at 24.4, 2.1% at 42.6 ng/L, and 0.9% at 60 ng/L. Training sessions were completed on time and educational material circulated through the hospital units and made available through the electronic laboratory handbook. The implementation was done in 2 steps: first phase targeted the Rapid Response Lab performing over 5,000 Troponin tests a month serving the critical care areas. A second phase, 6 weeks later, added the Core Lab supporting the inpatient non-critical units and the outpatient clinics.
Conclusion:
Manufacturer's claim for assay performance was successfully verified. The imprecision of the assay was acceptable at the established decision limits rule out (<6 ng/L) and a delta of 3 ng/L on serial samples. A real-world pre-implementation scenario was conducted where samples were assayed for both conventional and 5th generation hs-TnT to assess potential impact on patients, and on current and new protocols. This provided the basis for educational material needed to support the implementation.

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Performance Evaluation and Method Comparison of Atellica IM High Sensitivity Troponin I Assay

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Background: The objective of this study was to verify the analytical performance of the Siemens Healthineers Atellica® IM TnIH Assay* on the Atellica® IM 1600 Analyzer, and perform method comparison vs. Roche Cobas® e411 HS-TnT assay, ADVIA Centaur® High-Sensitivity Troponin I (TNIH) Assay, and Dimension Vista® High Sensitivity Troponin I (TNIH) assay. The effects of hemolysis and biotin were assessed.
Methods: Precision studies were performed per EP05-A3 and EP15-A3 using three levels of controls. A total minimum of 40 replicates were generated per sample. Limit of Blank (LoB) and Limit of Detection (LoD) were evaluated per EP17-A2. Linearity verification was performed (EP06-A). Method comparison was performed (EP09-A3). Serial measurements were obtained for lithium heparin samples from >50 chest pain ED patients. Troponin at admission and up to 6 h later were measured using the Atellica IM TnIH Assay, Roche Cobas e411 HS-TnT assay, ADVIA Centaur TNIH assay, and Dimension Vista TNIH assay. Hemolyzed samples (150 mg/dL and 500 mg/dL) and samples spiked with biotin (30 and 1500 ng/mL) were run in duplicate on the Atellica IM TnIH Assay.
Results: Precision results agreed with the manufacturer's claims: Within run repeatability CV%(SD)s for concentrations of 92, 242, and 5390 ng/L were 4,3(3.99), 3.5(8.49), and 3.6(195.2); within lab (total) CV%SDs were 5.4(4.97) 5.3(12.78), 5.1(272.38), respectively. LoB and LoD agreed with the manufacturer's claims of 0.58 ng/L and 1.27 ng/L, respectively. Method comparison of Atellica IM TnIH Assay vs. Cobas® e411 HS-TnT assay showed a Deming regression slope of 1.67 (95%CI 1.39 to 1.95) and intercept of -4.67 (95%CI -6.20 to -3.13) (n=118); Atellica IM TnIH Assay vs. ADVIA Centaur TNIH assay had a slope of 1.07 (95%CI 1.03 to 1.11) and intercept of 0.81 (95%CI 0.51 to 1.10) (n=127); and, Atellica IM TnIH Assay vs. Dimension Vista TNIH assay had a slope of 1.03 (95%CI 0.98 to 1.07) and intercept of -1.14 (95%CI -1.54 to -0.75) (n=122). All hemolysis (up to 500 mg/dL) and biotin (up to 1500 ng/mL) samples tested with the Atellica IM TnIH Assay demonstrated ≤10% change in results. Serial sample testing demonstrated good agreement among troponin assays in terms of trend between time points in patients where troponin was greater than the lower limits of the assay range. Discrepant results among high sensitivity assays involved patients where the difference between serial samples was <5 pg/mL.
Conclusion: The Atellica IM TnIH Assay has demonstrated good precision for detecting low cardiac troponin I concentrations and good correlation with the ADVIA Centaur and Dimension Vista TNIH assays. At levels of biotin up to 1500 ng/mL and hemolysis up to 500 mg/dL, there was ≤10% change in results.
* Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis.

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LC-MS quantification of BNP in plasma without immuno-enrichment

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Background:
B-type natriuretic peptide (BNP) is a cardiac hormone routinely measured in clinical laboratories to rule-out acute heart failure, and for screening or prognosis of heart failure. However, interpretation of BNP levels is not useful as a "rule-in" marker due to poor positive predictive value. The primary analytical technique used for BNP quantification in the clinic is the immunoassay, which is reported to show significant discrepancies among platforms due to several factors, including non-specificity of antibodies, heterogeneity of patient proteoforms, and patient phenotype. As yet, no attempts have been made to harmonize or standardize these clinical immunoassays through use of reference standards and the development of higher order approaches such as isotope dilution mass spectrometry (ID MS). BNP is found at exceedingly low serum concentrations, justifying an immuno-enrichment step prior to detection. One recent report detailing an antibody-free approach to quantifying plasma-spiked synthetic BNP allows for the possibility of achieving absolute quantification of BNP from matrix. In addition to abundance, stability is also a major concern for BNP quantification as the active form BNP_{1-32} is known to metabolize through numerous biochemical and enzymatic routes. At least 15 metabolites of BNP_{1-32} are known in blood of various chain lengths. This heterogeneity constitutes a major contribution of immunoassay variability. Here, isotope-labeled standards and LC-MS techniques have been used to measure BNP_{1-32} and its metabolites and estimate their stabilities in plasma. Further efforts have been made detect pre-spiked BNP at clinically-defined "healthy" levels using mass spectrometry in both intact and *in vitro* digested BNP, as well as native BNP_{1-32} in "diseased" patient samples.
Methods:
Isotopically-labeled and non-labeled synthetic, intact BNP standards were used for relative quantification of BNP metabolites using a targeted LC-MS/MS (MRM) approach. Isotopically-labeled and non-labeled synthetic tryptic peptides of BNP_{1-32} were used for estimating "total" BNP through a bottom-up technique, also based on LC-MRM-MS. An organic extraction pre-enrichment method and cleanup was optimized to measure BNP_{1-32} in clinically-obtained patient sera. This antibody-free approach permits use of an appropriate calibration system for the higher-order quantification of BNP.
Results:
An LC-MS/MS MRM method was optimized for quantification of BNP_{1-32} and 14 metabolites. BNP_{1-32} was shown to degrade rapidly in plasma (< 1% remaining after 2 hours). Other metabolites exhibited interesting kinetics, growing-in and degrading at various rates. Interestingly, "shared" tryptic peptides summed from all BNP metabolites were demonstrated to decrease slowly in abundance over time, suggesting possible unknown routes of BNP degradation. Synthetic BNP pre-spiked and extracted from plasma was detected down to <100 attamoles by LC-MS techniques, adequate for quantification at clinical levels. Native BNP

from patient sera was subsequently tested using the optimized LC-MS approach.
Conclusion:
Extending an antibody-free ID MS approach to the quantification of native BNP in plasma is necessary for development of the appropriate calibration system and measurement standards required to harmonize clinical immunoassays.

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Susceptibility of High Sensitivity Cardiac Troponin I and Gen 5 cTnT Assays to Biotin Interference

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Background: The FDA has alerted clinicians, laboratory personnel, and manufacturers of immunoassays that patient ingestion of high levels of biotin in dietary supplements can cause clinically significant incorrect lab test results. Depending on a test assay's configuration, increased biotin in patient samples can cause falsely high or low results. Our objective was to examine the susceptibility of two cardiac high-sensitivity troponin (hs-cTn) assays used globally in clinical practice to biotin supplementation. Methods: Four experiments were performed using excess, discarded lithium heparin plasma from patients with a positive cTnT (Roche 4th Gen) concentration. Overall, 133 specimens were analyzed by both the Abbott (investigational in US) hs-cTnI, Architect i2000), and Roche Gen 5 cTnT (cobas e601; FDA version of hs-cTnT used globally) assays. First, positive cTnT specimens (n=16) were titrated against a range of biotin levels (0-140 ng/mL) by simulating 1, 2, and 4-hour post-dose plasma biotin levels achievable for daily dose of 10 mg taken for 5 days. Second, the effect of mega doses of biotin (500 and 1000 ng/mL) was tested (n=10). Third, biotin levels were titrated against a range of biotin levels between 0 and 2000 ng/mL (n=12). Fourth, the effectiveness of streptavidin beads in blocking biotin that had been spiked into patient samples (n=3) was tested. Spiked samples with known levels of biotin (100 and 500 ng/mL) followed by blocking with 50 µL of streptavidin beads. The samples were then incubated at room temperature for 1 hour with intermittent shaking, centrifuged, and the supernatant was taken for cTn testing. False decreases at >10% or suppression of values below the 99th percentile URL (Gen 5 cTnT 16 ng/L, hs-cTnI 18 ng/L) in the presence of biotin were considered significant. Results: hs-cTnT concentration suppression crossed the 10% threshold at a 35 ng/mL biotin level. hs-cTnT concentrations were suppressed 24%, 50%, 78% and >90% at 50, 100, 140 and 500 ng/mL biotin levels, while hs-cTnI concentrations were <8% suppressed at all levels. 4%, 25%, 43% and 62% of hs-cTnT levels, respectively, were suppressed from increased to below the 99th percentile URL. Blocking with streptavidin beads eliminated hs-cTnT concentration suppression from 59% at 100 ng/L biotin and 95% at 500 ng/mL biotin levels to <7%. Conclusions. The Gen 5 cTnT assay that uses a sandwich immunoassay with biotinylated antibodies experienced significant negative interference with biotin concentrations at >35 ng/mL, that could result in false negative concentrations. The hs-cTnI assay was free from biotin interference at all concentrations tested.

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Highly Sensitive Cardiac Troponin Assay: Experience at a US Academic Medical Center

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Background: About 6 million patients present to the Emergency Department (ED) each year with complaints of chest pain. Of those, about 1.5 million rule in as having an acute myocardial infarction (MI). A key component of diagnosis is the rise and fall (>20%) of cardiac troponin (cTn), a highly sensitive and specific biomarker. A highly sensitive generation 5 (gen5) cTn assay has been in use in Europe and parts of Asia for over 10 years, but this was not clinically available in the United States. Recently, the FDA approved the gen5 assay for use within the U.S. In this study, the performance of a generation 4 (gen4) cardiac troponin T (cTnT) assay was compared to the highly sensitive gen5 cTnT assay for the first time within a U.S. healthcare system. **Methods:** Over a two-month period, all patients within University of California, San Diego (UCSD) Health in whom cTnT was ordered had both gen4 and gen5 measured. A total of 4,809 troponin orders from 2,516 patients (1,185 female and 1,221 male) were analyzed, giving an average of 1.9 troponin orders per patient. **Results:** The correlation between gen4 and gen5 (R^2) was 0.99 and 0.98 for male and female patients, respectively. The gen4 cTnT assay detected cTnT levels down to 0.01 ng/mL whereas the highly sensitive gen5 assay detected levels down to 0.006 ng/mL (6 ng/L). This increased sensitivity allowed for the detection of cTnT in 81% of samples (3879/4809) analyzed by the gen5 assay, whereas gen4 only detected cTnT in 33% of samples (1572/4809). Any detectable level of cTnT (> 0.01 ng/mL) using the gen4 assay was considered elevated. When using the gen5 sex-specific 99th percentile cutoffs of 14 ng/L for females and 22 ng/L for males, a total of 871 samples from 565 patients were positive by the gen5 assay but had undetectable levels by gen4. Many of these patients (302) had only a single troponin order, while 167 had at least a second cTnT ordered within 8 hours of the initial measurement. Of these 167 patients, 119 exhibited a stable elevation of cTnT by gen5 and 35 were detected by both gen4 and gen5 in the serial measurement. A total of 13 patients, however, exhibited >20% change in gen5 while remaining undetectable in gen4. Of these cases, 3 patients were diagnosed with a non ST-elevation MI (NSTEMI) and 2 with an ST-segment elevation MI. Two of the NSTEMI patients demonstrated a >20% elevation in cTnT using the gen5 assay before it was detected by gen4. **Conclusions:** The initial experience at UCSD Health was that gen5 cTnT detected elevations of cTnT in significantly more patients than the gen4 assay. The majority of these patients had stable elevations of cTnT. During this two month evaluation, gen5 cTnT detected a >20% changes in cTnT prior to detection by gen4 in several patients. These results confirm the importance of monitoring serial changes in time when implementing highly sensitive troponin assays and suggest that gen5 cTnT will allow for a faster rule-out protocol.

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Quantifying the prevalence of elevated biotin in a cohort with suspected acute coronary syndrome

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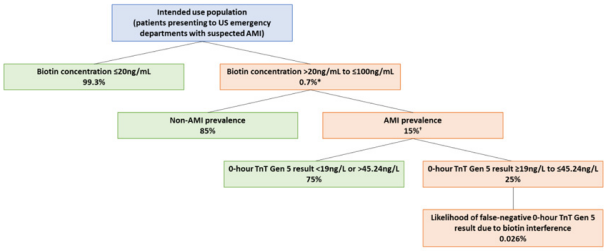
Background: Biotin can reduce recovery of the Elecsys® Troponin T Gen 5 (TnT Gen 5) assay at concentrations >20ng/mL (90% recovery), potentially leading to false-negative prediction of acute myocardial infarction (AMI). We aimed to determine the prevalence of biotin concentrations >20ng/mL and the 99th percentile biotin concentration in the intended use population. **Methods:** Biotin was quantified using an in-house assay (lower limit of detection: 0.1ng/mL) in residual 0-hour and 3-hour blood samples from 850 patients presenting to 15 US emergency departments with suspected AMI from July 2014 to October 2015. Potential impact of biotin on the negative predictive value (NPV) of the TnT Gen 5 assay and likelihood of false-negative AMI prediction was estimated at biotin concentrations 3 times the highest observed concentration (per Clinical and Laboratory Standards Institute [CLSI] EP07). **Results:** The 99th percentile biotin concentration for 0-hour samples was 2.62ng/mL and for 3-hour samples was 2.38ng/mL. These values are >7 times lower than the TnT Gen 5 assay interference threshold (conforming with CLSI EP07 criteria). Biotin was >20ng/mL in 1/797 (0.13%; 95% confidence interval [CI] 0-0.70%) 0-hour and 1/646 (0.15%; 95% CI 0-0.86%) 3-hour samples (30.23ng/mL and 24.48ng/mL, respectively); both samples were from the same patient. Based on extreme biotin assumptions derived from the study population (0.7% prevalence of 0-hour biotin up to 100ng/mL; maximal reduction in troponin recovery of 42% at 100ng/mL; 15% prevalence of AMI), 0-hour TnT Gen 5 results between 19ng/L and 45.24ng/L could potentially lead to false-negative AMI prediction. As 25% of patients with AMI had 0-hour results within this range, the likelihood of false-negative results due to biotin interference was estimated as 0.026% (**Figure**). **Conclusion:** Our results suggest biotin interference has a minimal effect on the NPV of the TnT Gen 5 assay and should not change current clinical practice.

Figure. Estimating the probability of false-negative AMI prediction, based on 0-hour TnT Gen 5 result, due to biotin interference



*Interference model assumed 0-hour biotin concentrations of 100ng/mL, approximately 3 times the highest observed concentration (30.23ng/mL); prevalence of 0-hour samples with biotin >20ng/mL was based on the upper confidence limit of the observed prevalence (0.13% [95% CI 0–0.70%]). †In the primary TnT Gen 5 study (Peacock et al. JAMA Cardiol 2017), 10.3% of patients were diagnosed with AMI; a more conservative estimate for AMI prevalence of 15% was used for this analysis.

A-106

Baseline High-Sensitivity Cardiac Troponin I Aids in Risk Assessment in Patients with Diabetes, Hypertension, and Dyslipidemia without Myocardial Infarction

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Background: Cardiac troponin has been shown to be a powerful prognostic biomarker for patients both with and without acute coronary syndromes. The objective of our study was to determine use of baseline high-sensitivity cardiac troponin I (hs-cTnI) concentrations for the risk stratification of patients with diabetes, hypertension, and dyslipidemia among emergency department patients without acute myocardial infarction. Methods: Prospective, observational cohort study (UTROPIA) including patients presenting to a United States emergency department in whom high sensitivity hs-cTnI concentrations were measured on clinical indication using an investigational assay (Abbott, 99th percentile URLs: males 34 ng/L and females 16 ng/L). Patients with myocardial infarction (n=168) were excluded. We assessed the impact of comorbidities across the entire cohort for each hs-cTnI tertile. Outcomes examined were 180-day mortality and major adverse cardiac events (MACE). Results: Among 1,463 patients, 436 (30%) had diabetes, 947 (65%) had hypertension, and 612 (42%) had dyslipidemia. Tertile analysis of baseline hs-cTnI concentrations showed a higher risk for 180-day mortality and MACE, with increasing risk by tertile (T) for each comorbidity examined; diabetes (mortality: T1: 2.8%, T2: 3.3%, T3: 10.6% and MACE: T1: 4.2%, T2: 5.3%, T3: 21.8%); hypertension (mortality: T1: 2.9%, T2: 5.2%, T3: 9.7% and MACE: T1: 4.9%, T2: 6.4%, T3: 21.5%); dyslipidemia (mortality: T1: 2.0%, T2: 6.0%, T3: 10.5% and MACE: T1: 3.5%, T2: 8.9%, T3: 23.5%). Cumulative comorbidities increased the risk for 180-day mortality and MACE in the entire cohort (mortality: no comorbidities: 3.7%, 1 comorbidity, 5.0%, 2 comorbidities: 6.4%, 3 comorbidities: 6.4% and MACE: no comorbidities: 4.5%, 1 comorbidity 8.8%, 2 comorbidities 11.1%, 3 comorbidities 13.9%). In patients with very low concentrations (T1: hs-cTnI <3ng/L), mortality and MACE rates were 0.5% when no comorbidities were present compared to 1.6% and 3.2%, respectively, when at least one comorbidity was present. Conclusions: Baseline hs-cTnI concentrations aid in the risk assessment of patients with diabetes, hypertension, and dyslipidemia, even without myocardial infarction; patients with higher hs-cTnI concentrations are at higher risk than those with lower concentrations. The cumulative presence of comorbidities increased the risk of adverse events and the presence of ≥1 comorbidity increased the risk for adverse events even in those with very low hs-cTnI concentrations.

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Sex-Specific 99th Percentiles Derived from the AACC Universal Sample Bank for 8 High-Sensitivity Cardiac Troponin Assays

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Introduction: Defining the 99th percentile upper-reference limit (URL) of cardiac troponin (cTn), the concentration threshold used to support the diagnosis of acute myocardial infarction (Universal Definition of Myocardial Infarction), is critically important. An added challenge is defining normality of subjects used to derive the 99th percentile URL. With increasing implementation of high sensitivity (hs)-cTnI and hs-cTnT assays in global clinical practice, clear guidance is needed to define normal (healthy) subjects used to determine 99th percentiles. Our objective was to determine overall and sex-specific 99th percentiles in 8 hs-cTn assays using a universal sample bank. Methods: Plasma specimens from apparently healthy subjects were obtained from the American Association of Clinical Chemistry (AACC) Universal Sample Bank (USB). These subjects included 423 men and 415 women who were screened using a health questionnaire. hs-cTnI (7 assays) concentrations were determined on: Abbott Architect i2000, Singulex Clarity, Beckman Coulter Access 2 Immunoassay System, Siemens Healthineers Dimension Vista 1500, Siemens Healthineers ADVIA Centaur XP, Ortho-Clinical Diagnostics VITROS 3600 Immunodiagnostic System, and ET Healthcare Pylon analyzers. hs-cTnT measurements were determined on 1 Roche Diagnostics Cobas analyzer (e602 using Gen 5 reagents). Hemoglobin A1c (URL 6.5%), NT-proBNP (URL 125 ng/L <75 y, 450 ng/L ≥75y) and eGFR (60 mL/min), along with statin use assisted in verifying subject normality, and used as surrogate biomarker health exclusions. 99th percentiles were determined by the non-parametric, Harrell-Davis Bootstrap, and Robust methods. Results: Demographics: ages 19 to 91y; Caucasian 58%, African American 27%, Pacific Islander/Asian 11%, other 4%; Hispanic 8%, non-Hispanic 92%. 99th percentiles for all assays, before and after exclusion (decreased using surrogate excluders), were influenced by the statistical method use, both for the overall and sex-specific 99th percentiles, with substantially different 99th percentiles between assays. For all assays, men had higher 99th percentiles (ng/L) than women. For women, the Roche (21%) and Beckman (48%) assays did not measure cTn ≥LoD in >50% of subjects. Conclusions: Our study has important clinical practice implications, in that a) sex-specific 99th percentiles vary according to the hs-assay and the statistical method used, b) not all hs-cTn assays provide measurable concentrations ≥ LoD in >50% for women, and c) surrogate exclusion criteria used to define normality tends to lower 99th percentiles.

A-108

Poor correlation and concordance between NT-proBNP and BNP in patients with suspected heart failure

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B type natriuretic peptide (BNP) and N-terminal- pro B type natriuretic peptide (NT-proBNP) are viewed as comparable in their ability to diagnose and monitor heart failure in clinical guidelines. However, no large-scale study has directly established a correlation between BNP and NT-proBNP. This is particularly relevant in the context of chronic kidney disease (CKD), as NT-proBNP is elevated in CKD relative to BNP. Importantly, a large number of patients with CKD have concomitant heart failure. Our laboratory recently switched from BNP to NT-proBNP as the primary marker of ventricular strain. Prior to this change, we performed simultaneous BNP (Abbott BNP, Chicago USA) and NT-proBNP (Roche Corporation proBNP II, Basel Switzerland) testing for our clinicians for two months. We then compared plasma concentrations of BNP and NT-proBNP in different patient populations including those with and without CKD as determined by the CKD-EPI equation. Patient location, age, sex and creatinine along with BNP and NT-proBNP concentrations were available for 2,729 patient samples. The primary analyses were correlation and diagnostic concordance between BNP and NT-proBNP in the presence or absence of CKD in the acute (i.e. patients seen in the Emergency Department) vs. non-acute settings (all other settings) and at different ages. Common cutoffs for BNP and NT-proBNP to rule out or rule in acute and non-acute heart failure were used. In the acute ED setting, overall concordance between BNP and NT-proBNP was 72.0% with a weighted kappa of 0.695. In non-acute patients, the overall concordance was 92.5% with a kappa of 0.642. Concordance was not statistically different between different age groups. Patients

with an eGFR <60 ml/min had significantly lower concordance (70%, kappa 0.636) than those with eGFR >60 ml/min (77%, kappa 0.731). Moreover, the mean ratio of NT-proBNP to BNP was significantly higher in patients with CKD (10.7:1) than in non-CKD patients (5.7:1). Consequently, there was significantly greater correlation between NT-proBNP and BNP concentrations in patients with eGFR >60 (r^2 = .717) than patients with eGFR <60 (r^2 = .581) in the acute setting. Finally, for patients with multiple measurements of natriuretic peptides, there was variability between changes in BNP relative to changes in NT-proBNP concentrations over time. Overall, 20% of paired temporal measurements had an inverse relationship (increase in one peptide and a decrease in the other). Together these data showed surprising differences in diagnostic concordance and monitoring values between BNP and NT-proBNP, particularly among patients with CKD. We conclude that using the current cutoffs for heart failure, concentrations of NT-proBNP and BNP have surprisingly poor diagnostic concordance. Further studies are required to examine the diagnostic concentrations of natriuretic peptides, modes of clearance, and assay specificity for the multiple circulating forms of natriuretic peptides.

A-109

Analytical Comparison of High Sensitivity Cardiac Troponin I and T Assays in Patients Presenting to the Emergency Department - the CONTRAST Study

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Background: This study compared the frequency of increases in high-sensitivity cardiac troponin (hs-cTn) I, hs-cTnT, and contemporary cTnI assay performances in patients being evaluated in a US emergency department undergoing cTnI measurements on clinical indication. Objectives were to determine the concordance of positive and negative results based on sex-specific 99th percentile upper reference limits (URLs) and correlations between hs-cTn assays and a contemporary assay. Methods: This analytical sub-study examined plasma (EDTA) specimens (n=1,000) randomly selected from >9,000 specimens from patients enrolled in the 'COmparisoN of High-sensitivity Cardiac TRoponin I and T Assays' (CONTRAST) study (clinicaltrials.gov NCT03214029). Patient clinical information was not included nor was available to determine whether single or multiple serial specimens from the same patient were included. Fresh specimens were measured by the hs-cTnI Abbott Architect assay and the Gen5 cTnT Roche cobas e601 assay directly upon completion of clinical testing with the contemporary cTnI (Abbott). Two sex-specific 99th percentiles were used to evaluate each hs-assay: manufacturer's package inserts (PI) and 99th percentile URLs derived from the AACC's Universal Sample Bank (USB), were: Abbott, PI - M 34 ng/L, W 16 ng/L; USB - M 19 ng/L, W 10 ng/L; Roche PI - M 22 ng/L, W 14 ng/L; USB M 16 ng/L, W 10 ng/L. The 99th percentile URL of the contemporary assay was 0.03 µg/L. The values of all assays were plotted and described using Pearson's correlation coefficients (r value) with Fisher's 95% confidence intervals (CI). Proportions of increased results were cross-tabulated to determine agreement with the Kappa statistic. Results: Using PI URLs, the percentage of specimens above the 99th percentile was 30% for hs-cTnI and 47.4% for Gen 5 cTnT. For comparison, using the lower USB URLs increased the percentage of increased results to 40.6% for hs-cTnI and 55.5% for Gen 5 cTnT. Using the PI URLs, 94.3% of Gen 5 cTnT results were greater than the 99th percentile in specimens with increased hs-cTnI results. In comparison, only 59.5% of hs-cTnI results were increased in specimens that had increased Gen 5 cTnT results; kappa of 0.575 (95%CI, 0.507,0.623). A similar difference in cross-assay increased rate was also observed using the USB URLs. Compared to a rate of increased contemporary cTnI results of 31.1%, using the PI hs-assays URLs, there was a 3.6% decrease in hs-cTnI increases compared to a 52.4% increase in Gen 5 cTnT increases. The Pearson correlation coefficient between the hs-cTnI and hs-cTnT assays was 0.298 (CI 0.241,0.354). Conclusions: Our findings demonstrate substantial differences between the hs-cTnI and Gen 5 cTnT assays in the proportion of values above the sex-specific 99th percentiles, regardless of the URL used. There were greater numbers of increased values for both assays using the lower USB 99th percentile URLs, and for Gen 5 cTnT for both URLs compared to the hs-cTnI assay. Furthermore, there was a substantial increase in the proportion of increased values found between the contemporary cTnI assay and the Gen 5 cTnT assay; with a small decrease found for the hs-cTnI assay.

A-110

Race- and Sex- Dependent Association of BNP and Galectin-3 Levels with 6-Month All-Cause Mortality in Patients with Elevated BNP

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Background: B-type natriuretic peptide (BNP) and galectin-3 (Gal-3) are recognized as outcome-predicting factors for heart failure patients, with increased risks of death in the presence of low hemoglobin (Hb) or high creatinine. This study aims to evaluate the association of serum BNP and Gal-3 levels with 6-month prognosis of patients with elevated BNP. **Methods:** A total of 710 patients (ages 18 or older) from two medical centers with BNP>100 pg/mL at admission were enrolled in this study. A reflex testing of Gal-3 was then performed via the Abbott Architect immunoassay. Hb and serum creatinine concentrations at admission, the occurrences of re-admissions and death within 6 months from the first discharge were retrieved via patient chart review. The biomarker levels, all-cause death rates, days to re-admissions, and re-admission frequencies were compared among different races (white/black), sexes (male/female), and quartiles groups based on BNP and/or Gal-3 levels. The relationship between biomarkers and mortality was assessed via correlation and multivariate regression analysis (MRV). **Results:** Black patients had significantly higher levels of Gal-3, creatinine, and Hb than white patients. Male patients had greater levels of Gal-3 and Hb than female patients. However, similar BNP levels, death rates, days to re-admissions, and re-admission frequencies were observed in patients with different races or sexes. When patients were divided into 4 subgroups according to the quartiles of their BNP levels, significantly higher death rates (2-5 times) were only observed in white or male patients in the highest BNP quartile (Q4:>936.6-21375.1 pg/mL vs. Q1: 101.2-196.4 pg/mL). Additionally, the 30-day and 6-month re-admission frequencies were 2 times higher in BNP Q4 than Q1 in white and black patients, respectively. On the contrary, death rates of patients in higher Gal-3 quartiles (Q3: > 26.0-38.3 ng/mL and Q4: >38.3-180.1 ng/mL) were 2-10 times greater than those in Q1: (8.4-19.0 ng/mL) irrespective of race and sex. Patients with higher Gal-3 (Q3 or Q4: vs. Q1) also had a higher 30-day readmission frequency, with no difference between different races or sexes. No additional increase in mortality or re-admission rates was observed when BNP and Gal-3 quartiles were combined. Correlation analysis revealed positive association between death and creatinine in white females (R = 0.254), but negative correlation with Hb in black males (R = -0.264). Moreover, Gal-3 showed strong positive correlation with creatinine (R = 0.571), weak positive association with BNP (R = 0.275), but negative association with Hb (R = -0.302) in all patients. MRV analysis showed that Gal-3 was the only significant variable in the all-cause mortality of all patients, which together with creatinine and Hb formulate a linear regression to predict the death in black male patients (Mortality = 0.005XGal-3 - 0.023XHb - 0.033XCreatinine, $p<0.05$). In contrast, BNP was only associated with the mortality in white patients ($p = 0.018$). **Conclusion:** Our data suggest the race- and sex- dependent association between BNP and Gal-3 with 6-month all-cause mortality in patients with elevated BNP. In addition to BNP, Hb and creatinine, Gal-3 measurement may provide extra value for predicting all-cause mortality, especially in black male patients.

A-111

A Single High Sensitivity Cardiac Troponin I Measurement From Siemens Healthineers Can Be Used to Rule Out Acute Myocardial Infarction at Low Risk in Patients Presenting to the Emergency Department

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Background: High sensitivity cardiac troponin (hs-cTn) assays are able to quantitate low concentrations of cTn and provide an opportunity to rule out acute myocardial infarction (MI) at an early stage following a patient's presentation to an emergency department. The objectives of the current study were to examine the performance of single hs-cTnI measurement strategy to rule out acute MI and predict 30-day safety outcomes at presentation in these patients. Methods: This was a prospective, observational study of patients (n = 2333). Acute MI occurred in 299 patients (12.8%). Patients presented to US emergency departments with suspected acute coronary syndrome. Cardiac troponin measurements

were obtained using the investigational ADVIA Centaur XPT TNIH (hs-cTnI) Assay (Siemens Healthcare Diagnostics Inc.). Clinical data and hs-cTnI results were analyzed to determine: 1) clinical sensitivity and negative predictive value (NPV) for ruling out acute MI and 2) safety outcomes of acute MI and death at 30 days, using the hs-cTnI limit of detection (LoD) 1.6 ng/L concentration. Results: In patients with a hs-cTnI <LoD (n=376, 16.1%), the clinical sensitivity and negative predictive value (NPV) for acute MI were 100% (95% CI 98.8,100) and 100% (CI 99.0,100), respectively. Further, the sensitivity and NPV for the safety outcome of acute MI or death within 30 days for hs-cTnI <LoD were 99.7% (CI 99.1,100) and 99.7% (CI 99.2,100), respectively. One patient out of 376 (0.26%) had an event within 30 days. Conclusion: A strategy of using a single hs-cTnI <LoD at presentation allowed the immediate identification of 16.1% of patients highly unlikely to have acute MI and who were at very low risk for events at 30 days. Additional study to understand the clinical utility and cost-savings of this strategy is needed.

A-112

Red Cell Distribution Width and Cardiovascular Risk: four-year follow up of Longitudinal Study of Adult Health (ELSA-Brasil)

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Background: Red Cell Distribution Width (RDW) is a quantitative laboratory test that measure the variability in the size of circulating erythrocytes. RDW is easily obtained with automated hematology analyzers, as part of complete blood count (CBC), and is generally used as an indicator of the differential diagnosis of microcytic anemia. Recent studies have shown that RDW is a predictive, diagnostic, and prognostic marker of mortality and cardiovascular events in general population as well as in patients with cardiovascular diseases (CVD). Although pathophysiological mechanisms are still unclear, the evidence obtained so far encourages further research on the RDW in different populations and clinical settings. The aim of this study was to investigate the relationship of the RDW at the baseline of the study with the risk of CVD risk at four years of follow-up in participants of the Longitudinal Study of Adult Health (ELSA-Brasil).**Methods**: We used baseline (2008-2010) and second visit (2012- 2014) data of 4471 civil servants enrolled in the ELSA-Brasil cohort. Mixed linear regression model for longitudinal data was used to determine association between RDW and increased cardiovascular risk based on Framingham Risk Score (FRS). RDW were quantified by coefficient of variation of red blood cells volume (RDW-CV%) using XE 2100 D hematologic analyzers (Sysmex, Kobe, Japan), that use impedance technology to estimate particle count and volume. The population was distributed according to their exposure to different risk factors, and stratified for cardiovascular risk, based on FRS.**Results**: RDW (adjusted r^2=0.921; $p<0.001$) was independently associated with the FRS after adjustment for education, skin color, body mass index, abdominal waist circumference, bariatric surgery, hemoglobin concentration, mean corpuscular volume, platelets, C-reactive protein, alcohol consumption. It was observed that a one-unit increase in RDW increases the FRS by 14%, in average. **Conclusion**: In this large cohort of free living Brazilians, ours results showed that RDW is independently associated with increased CVD risk based on the FRS at four-year follow up. The RDW, an inexpensive, easily obtained, and widely used test, holds potential evidence to be a novel biomarker in predicting CVD risk in asymptomatic individual. Prospective follow-up of ELSA-Brasil cohort is necessary to confirm the association between RDW and CVD.

A-113

Assay Development And Evaluation Of Serum Aggrecan And Versican As Novel Biomarkers For Thoracic Aortic Aneurysm And Dissection

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Objectives: Thoracic aortic aneurysm and dissection (TAAD) is a progressive vasculopathy with a high rate of mortality due to an increased risk of vessel wall rupture. Aorta diameter is currently the gold standard for dictating surgical intervention, however the majority of dissections occur below surgical decision limits, illustrating a pressing need for new biomarkers for aneurysm detection and dissection risk stratification. Proteoglycan accumulation in medial degeneration lesions is a histologic hallmark of TAAD. We hypothesize that the proteoglycan constituents of medial degeneration lesions will enter the circulation in aneurysm and/or dissection and can be used as diagnostic and prognostic biomarkers. The objectives of this study were to develop assays for the detection of proteoglycans and evaluate their presence in the peripheral circulation of TAAD patients. **Methods**: We identified the proteoglycan constituents of the ascending aorta by tandem mass spectrometry (MS/MS) analysis of affinity-isolated proteoglycans. Ascending aorta tissue was collected from patients undergoing elective (aneurysm) or emergent (dissection) surgical intervention. Aortas from heart donors were used as normal controls. Total protein was extracted from tissue and proteoglycans isolated by anion exchange chromatography. Shotgun MS/MS was performed on isolated proteoglycans using a Thermo Scientific Orbitrap Elite hybrid analyzer. Shotgun analysis was repeated on isolated proteoglycans as well as on total aorta protein extracts using a Thermo Scientific Orbitrap Fusion Lumos tribrid analyzer to identify peptide candidates for selected reaction monitoring (SRM) assay development. Targeted analysis was performed on TAAD and control serum samples to verify candidate peptides could be identified in the peripheral circulation. Additionally, proteoglycan concentration in TAAD patient serum was determined in triplicate by a commercially available sandwich ELISA (research use only; R&D Systems). Blood from TAAD patients (n = 25) was collected pre-operatively in 3.5 mL SST BD vacutainer tubes. **Results**: The proteoglycans aggrecan and versican were identified as major constituents of medial degeneration lesions. Due to the large number of post-translational modifications in the central glycosaminoglycan domains, peptides were limited to the N- and C-terminal globular domains and included 11 and 18 unique peptides for aggrecan and versican, respectively. Two peptides for each proteoglycan were chosen for further SRM development. Targeted MS/MS analysis of TAAD serum identified peptides, but at low intensities, suggesting further pre-analytic processing, such as albumin/Ig depletion, may be required. An aggrecan ELISA was optimized for serum with a sensitivity of 100 pg/mL and an intra-assay imprecision of <5.0 %CV (range: 0.0-16.5%). 11 of 25 TAAD patients had detectable aggrecan levels (>100 pg/mL), including 3 of 5 dissection patients, with concentrations ranging from 279-17979 pg/mL. **Conclusions**: Aggrecan and versican accumulate in TAAD and are detectable in the peripheral circulation by MS/MS and immunoassay. Despite consistent detection by MS/MS, serum aggrecan levels were detectable by ELISA in some, but not all cases of TAAD indicating that differences in circulating fragments may be influenced by disease progression, primary etiology, and other unknown factors. Aggrecan and versican are potential serum biomarkers for TAAD and warrant further investigation.

Tuesday, July 31, 2018

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

Clinical Studies/Outcomes

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Determination of bioenergetic defects in mitochondrial disease patients using extracellular flux analysis

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Background: Mitochondria control cellular homeostasis through maintaining proper bioenergetic programs. Defects in mitochondrial function arising from mutations in mitochondrial or nuclear genome, (mitochondrial diseases), can be presented with a wide specrum of neurological, muscular and cardiac symptoms. The multi-system involvement and the heterogeneus fetures of these defects mimic neurological and sytemic diseases that make diagnosis often challenging. The influence of genetic, environmental, lifestyle factors and age on mitochondrial activity further complicate the diagnosis. Use of invasive muscle biopsies provide only limited information and genomic analysis will not assess mitochondrial function. In this study we report the development and validation of a panel of minimally invasive mitochondrial assays that can assist diagnosis of mitochondrial diseases. Methods: This test utilizes the concept that circulating leukocytes and platelets can act as sensors or biomarkers of bioenergetic dysfunction that occurs in mitochondrial diseases. Monocytes, lymphocytes and platelets were isolated from healthy subjects and mitochondrial diseases patients using a protocol involving density gradient centrifugation and magnetic bead-based purification (MACS technology). Using the extracellular flux analyzer the oxygen consumption rates of mitochondria in intact monocytes and platelets are measured employing two different protocols (1) the mitochondrial stress test and (2) mitochondrial respiratory complex activities. The mitochondrial stress test will determine the bioenergetic parameters in intact cells (basal, ATP-linked, proton-leak, maximal, reserve capacity and non-mitochondrial respiration) which will be used to calculate the health of the mitochondria termed as the bioenergetic health index (BHI). For mitocxhondrial complex activity the plasma membrane is selectively permeabilized without altering the mitochondrial membrane and the maximal activity of mitochondrial respiratory enzyme complexes (Complex I, Complex II and Complex IV) are determined in the presence of specific metabolic substrates. Results: The results show that there is significant loss of specific mitochondrial complexes (Complex I, Complex II or Complex IV) exist in mitochondrial disease patients. The cellular bioenergetic parameters were also showed significant decrease in mitochondrial disease patients. The bioenergetic and mitochondrial profiles demonstrate a high degree of heterogeneity in specific defects among mitochondrial disease patients. Conclusion: This suggests that the mitochondrial assays have the potential to determine bioenergetic defects in mitochondrial diseases.

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Traumatic Brain Injury: Combining GFAP and UCH1-L1 Serum Biomarkers Predicts Head CT Outcomes

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Background: Traumatic Brain Injury (TBI) is caused by external force to the brain resulting in impaired cognitive and/or physical function. TBI causes ~3 million emergency department visits, hospitalizations, or deaths annually in USA. The standard of care for suspected TBI is neurological assessment followed by neuroimaging of the head by Computerized Tomography (CT), and exposure to substantial ionizing radiation. A blood test for TBI may reduce need for CT and would be very useful in military, sports, and traumatic injury applications. A test, coined Brain Trauma Indicator™ (BTI; Banyan Biomarkers), measures two brain-specific proteins, ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP), which are rapidly released into the bloodstream in TBI. We characterized the measurement and clinical performance of UCH-L1 and GFAP testing by BTI in suspected TBI patients. **Methods:** Testing was performed at 3 sites. UCH-L1 and GFAP were assayed by chemiluminescent ELISA using the BTI. Lower and upper limits of quantitation were 80pg/mL and 2560pg/mL for UCH-L1, and 10pg/mL and 320pg/mL for GFAP. Cutoffs are used for interpretation. Cutoffs (%CV) for UCH-L1 and GFAP were 327pg/mL (6.2%) and 22pg/mL (4.9%), respectively. If either or both biomarker(s) were above-cutoff, the result was Positive; if both results were below-cutoff, the result was Negative. Samples with Invalid or No Result measurements were re-tested so results for all subjects were reported. The BTI test was examined as a rule-out, so cutoffs were set to maximize sensitivity and Negative Predictive Value (NPV). The Figure shows Clinical Performance compared to CT. **Conclusions:** The BTI test's sensitivity was 97.5% in suspected TBI subjects; of the negative BTI results, very few were false negatives (NPV=99.6%). The high sensitivity and NPV support the clinical utility in an Emergency Department setting of the BTI to rule out the need for CT in subjects with suspected TBI.

Performance Characteristics, 6.2% Prevalence of CT[a] positive		
Sensitivity	97.5%	95% CI[b]: 92.9-99.5%
NPV[c]	99.6%	95% CI: 98.7-99.9%
LR[d] Negative	0.069	95% CL[e]: 0.170
Specificity	36.5%	95% CI: 34.2-38.7%
PPV[f]	9.2%	95% CI: 7.6-10.9%
LR Positive	1.534	95% CL: 1.468

[a]CT = computed tomography scan; [b]CI = confidence interval; [c]Negative Predictive Value; [d]Likelihood Ratio; [e]CL=confidence limit, [f]Positive Predictive Value

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Reference intervals for liver specific clinical chemistry parameters in apparently healthy nepalese adult population.

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Background: The reference interval (RI) is important for the screening, diagnosis, treatment, and monitoring of common disorders like liver diseases. During the last decades, the number of routine clinical chemistry tests has increased dramatically in Nepal. However, there are no valid RIs for any biochemical parameter in Nepal. RIs currently in use are those supplied by reagent manufacturers which are not derived from local population. Therefore, we conducted this study of establishing RIs of 25 major biochemistry parameters for Nepalese population, as a part of the IFCC global multicenter study on RVs. In this report, results of 7 parameters belonging to liver function tests are described. **Methods:** A total 617 apparently healthy individuals were recruited nationwide from five different regions of the country. Blood samples were collected and sera were separated and stored at −80^{0}C. All the samples were measured collectively by auto-analyzer AU480 (Beckman-Coulter).Test results were standardized by measuring the value-assigned panel of sera which was provided by IFCC committee on Reference Intervals and Decision Limits (C-RIDL). With application of the latent abnormal values exclusion (LAVE) method, reference intervals (RIs) were derived by both parametric and non-parametric method by use of Reference Master software provided by C-RIDL. **Results:** The reference intervals (lower limit−upper limit) derived for [MF] males[M] and females [F], were total protein MF (67−82),M:(67−81),F:(66−82) gm/L albumin MF:(41−52), M:(43−53), F:(40−51)gm/L; Total Bilirubin (TBil) MF:(2.7.21.9),M(2.6-23.5),F:(2.2-19.6) micromol/L; ALT MF(3−44)U/L, M:(4−57),F:(3−35)U/L;AST MF:(7−37), M:(8-41),F:(5−31) U/L;ALP MF:(131−339),M:(138−416), F:(113−355) U/L, γGT MF:(10−81),M:(11−107), F:(9−36) U/L. Furthermore, gender-wise evaluation showed prominent increase in ALP and γGT in females after 45 years of age. While albumin in males showed linear reduction with age. These variations may be attributed to smoking and alcoholic habits of male than females of this region..RI of total protein is shifted to the higher side and TBil and ALT are shifted to the lower side in Nepalese compared to those of other countries. **Conclusion: :** This is the first study to establish clinical reference intervals for healthy adult Nepalese population. Some of our RIs which were standardized based on the serum panel differ from those of other countries, indicating the importance of deriving country specific RIs.

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High sensitivity C-reactive protein and lipid profile in Hypertension

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Background:
Hypertension (HTN), a major public health problem worldwide has the prevalence of 33.8% in young adults in Nepal. Various studies have shown the coexistence of prevalence of HTN and dyslipidemia showing the adverse impact on the vascular endothelium resulting into enhanced atherosclerosis leading to cardiovascular diseases. High sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation and a strong predictor of future cardiovascular events is an acute phase reactant protein belonging to pentraxin family. Its level rises markedly after an acute inflammatory stimulus. hs-CRP levels <1 mg/L were considered low-risk, 1 to 3 mg/L as average risk, and >3 mg/L as high-risk for CVD. An early detection of the risk for cardiovascular diseases (CVDs) is essential to prevent the future cardiovascular events. Hence, the objective of this study is to determine the relationship between blood pressure, hs-CRP and lipid profile in hypertensive as well as in normotensive participants and to detect the risk for CVDs.
Methods:
Forty seven newly diagnosed cases of hypertension and fifty age and sex matched healthy controls with prior informed consent were enrolled. The patients were clinically diagnosed as hypertensive according to the recommendation by Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7). Patients with chronic inflammatory diseases such as rheumatoid arthritis (RA), autoimmune diseases, tuberculosis and any previous history of diabetes/ stroke etc. were excluded from the study. The anthropometric measurements were done to calculate Body Mass Index (BMI), hs-CRP was measured by ELISA method and lipid parameters were estimated by spectro-photometric method in fasting blood sample. Non-HDL-C was calculated by subtracting high density lipoprotein cholesterol (HDL-C) from total cholesterol (TC). Data were analyzed by student 't' test, correlation was determined by Pearson correlation coefficient and P value was considered significant when $P<0.05$.
Results:
The result showed statistically significant increase in BMI (24.60 ± 3.72 Vs 22.55 ± 2.59), TC (178.27±37.14 Vs 146.28±26.31), LDL-C (108.46 ± 38.33 Vs 82.42 ± 20.41), Non-HDL-C (136.74± 35.58 Vs 104.12± 26.02), and hs-CRP (3.21 ± 3.03 Vs 1.35 ± 1.27) in hypertension as compared to controls ($P< 0.05$). However, the increase in TG and the decrease in HDL-C were statistically not significant. Furthermore, hs-CRP and Non-HDL-C had positive correlation with BMI, Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) and was statistically significant ($P< 0.05$).
Conclusions:
Hypertensive subjects have significantly higher values of hs-CRP, non-HDL-C and BMI than that in controls. Even more, the levels of hs-CRP and Non-HDL-C are also correlated significantly with systolic as well as diastolic blood pressure. Hence, hs-CRP and non-HDL-C can be used as a marker for the risk for CVDs.

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Microalbuminuria in Chronic Obstructive Pulmonary Disease

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Background
Chronic Obstructive Pulmonary Disease is a global disease which results due to the irreversible airflow limitation and its epidemiology is in increasing trend. Up to date COPD is the second most common noninfectious disease and fourth leading cause of mortality in the world, causing approx. 2.75 million deaths annually and global mortality is predicted to be more than double by 2020. Microalbuminuria (MAB) is believed to reflect a state of generalized endothelial dysfunction, and therefore it is an emerging therapeutic target for primary prevention strategies in COPD patients and to rule out their cardiovascular risk. Thus, this is the first study done in Nepal to rule out the relation between microalbuminuria in COPD patients.
Objectives
To determine urine microalbumin in COPD patients and asymptomatic smokers.
Methods
This is case control study involving two hundred and forty nine patients diagnosed with COPD, and ninety eight asymptomatic controls (smokers) who were matched according to age and sex. They were selected from outpatient department (OPD) and In patients Department (IPD) of Respiratory and Critical care unit in Tribhuvan University Teaching Hospital (TUTH). Urine microalbumin, Arterial Blood Gas Analysis and Pulmonary Function Test were assessed in both groups. For comparison, COPD patients were divided into four different subgroups based on the duration of COPD. SPSS ver. 21.0 was used to analyze the data. Mean comparison was done by t-test. Group comparison was done by Chi-square test. Pearson's correlation was used to establish the correlation.
Results
Majority of patients with microalbuminuria (MAB) in this study were in the GOLD stage of I and II and rarely of stage III but stage IV was absent. Patients with COPD had significantly higher levels of microalbuminuria than control subject ; higher UACR ; lower FEV1 ; lower FVC and lower FEV1/FVC ratio with p=<0.001, higher PCO2 (p=0.010) and lower PH (p=0.028) respectively. Out of two hundred and forty eight COPD patients, two hundred ten were with MAB and higher UACR (P=<0.001), thirty eight patients were without MAB. COPD patients with MAB were more hypoxic and more hypercapnic compared to COPD patients without MAB but was statistically non-significant with p value of 0.172 and 0.313 respectively. To evaluate the impact of duration of occurrence of COPD, analysis was done by grouping the cases into four different continuous intervals according to duration of COPD (less than 5 years, 5 to 10 years, 10 to 15 years and more than 15 years). There were total of 248 cases among which 42 cases were below 5 years of duration having MAB (81.976±37.4175), 53 were in 5 to 10 years of duration having MAB (104.257±47.9539) , 77 were in 10 to 15 years of duration having MAB (104.955±40.44) and finally 77 were above 15 years having MAB (146.442±46.83) .
Conclusion
Microalbuminuria is found increased in patients with COPD compared to healthy smokers. Its level is also increased as the disease progresses in terms of duration. Microalbuminuria can be a clinically relevant tool identifying COPD patients with poor prognosis and their monitoring.

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Effects of the levels of vitamin D in serum and seminal plasma on sperm motility and morphology

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Background: Male factor infertility is responsible for about 25% of infertility causes. Sperm motility and morphology are the most important parameters in assessment of sperm functions. Although the mechanism of vitamin D (vit D) effect on male fertility has not been fully elucidated, possible mechanisms are being investigated in various studies.In these studies vit D receptors are detected on membrane of sperm and it is observed that vit D could increase the sperm motility by increasing the levels of intracellular calcium via vit D receptors.In this prospective study, it was aimed to investigate the possible effects of vit D levels in serum and preseminal fluid on male fertility by assessing the sperm parameters.
Methods: Samples of serum, sperm and preseminal fluid were collected from 100 men who applied to Hacettepe University Department of Obstetrics and Gynecology with infertility complains between September 2016 and October 2016. Sperm samples were assessed manually by the single-blind method according to WHO criteria in terms of sperm parameters.The levels of vit D in serum and seminal plasma were compared in terms of sperm parameters.
Results: There was not statistically significant difference between not only the levels of vit (p=0.463), but also the levels of intracellular calcium in serum and seminal plasma (p=0.878). Moreover, both total sperm motility(r=0.241) and progressive sperm motility (r=0.217) were found to be significantly correlated with the levels of seminal plasma calcium.
Conclusion: Any studies related to the comparison of the vit D levels and intracellular calcium in aforementioned fluids have not been found according to the current literature. As observed from our results, there was a significant positive correlation between the number of forward moving sperms, percentage of sperm in normal morphology and the levels of vit D. However, this research should be supported by studies that include more patients and compare the same parameters of healthy individuals.

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Genotype-based epigenetic factors in identical twins discordant for positive TgAb

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Background: Epigenetic factors associated with the development of autoimmune diseases are unclear. Monozygotic twin pairs discordant for positive anti-thyroglobulin autoantibodies (TgAb) are useful to examine the epigenetic factors because of their identical genetic background. To clarify the discordant epigenetic differences affecting the development of TgAb. **Subjects:** We selected subjects from 257 Japanese monozygotic twins, recruited from the registry established by the Center for Twin Research at Osaka University. TgAb positive concordant (PC) pairs were 5.7% (4 pairs) and 9.6% (18 pairs) of male and female pairs, respectively. TgAb discordant (DC) pairs were 11.4% (8 pairs) and 8.0% (15 pairs) of male and female pairs, respectively. TgAb negative concordant (NC) pairs were 78.6% (55 pairs) of male pairs and 74.3% (139 pairs) of female pairs. To perform stricter grouping, in this study, we set the cut-off value for positive TgAb to 50.0 IU/mL (TgAb Negative: <28.0IU/mL, TgAb Positive: >=50.0IU/mL. TgAb Borderline: =>28.0IU/mL and <50.0IU/mL). Nineteen discordant (6 male and 13 female pairs) and 185 concordant pairs (48 male and 137 female pairs) for TgAb positivity were finally examined. **Methods:** We evaluated DNA methylation levels of genomic DNA using the Infinium HumanMethylation450 BeadChip Kit (Illumina). We also genotyped gene polymorphisms using the Omni5-4 BeadChip Kit (Illumina) to clarify genetic background specific for discordant twins. **Results:** We did not find any CpG sites with significant within-pair differences of methylation levels in TgAb DC pairs after correction for multiple comparisons. However, 155 polymorphisms specific for TgAb DC pairs were significantly different in genotype frequencies from those of concordant pairs, and none of them was located on the HLA region of chromosome 6. In TgAb DC pairs with some specific genotypes of these polymorphisms, we observed four CpG sites exhibiting significant within-pair differences in each DC pair, even after correction for multiple comparisons. **Conclusions:** We found that the genetic background specific for TgAb DC twins who are susceptible to epigenetic changes are different from that specific for TgAb PC twins, and clarified the genotype-based epigenetic differences in TgAb-DC monozygotic twins.

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Performance Evaluation of the VERSANT HCV Genotype 2.0 Assay (LiPA) Using Manual and Automated PCR Setup Workflow on the VERSANT kPCR Sample Prep Instrument

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Background: The high degree of genetic diversity of the Hepatitis C Virus (HCV) poses a major challenge to its treatment. Determination of HCV genotype is needed to optimize HCV treatment type, dose, and duration to improve patient treatment outcome. The VERSANT® HCV Genotype 2.0 Assay (LiPA)* is a line-probe assay that identifies HCV genotypes 1-6 and subtypes a and b of genotype 1 in human serum or plasma specimens using reverse hybridization technology. The assay has a Limit of Detection (LoD) of 400 IU/mL in plasma and 650 IU/mL in serum. Previously, we have shown that the VERSANT HCV Genotype 2.0 Assay LiPA, using manual PCR setup, provides accurate determination of HCV genotypes 2, 3, 4, 5, 6, and subtypes 1a and 1b for optimal patient therapy (Abstract #B-089, AACC 2017). Automated PCR setup on the VERSANT kPCR Sample Prep instrument (SP) is now available for this assay and is evaluated here. **Methods:** The current LiPA extraction workflow requires the operator to manually pipette the PCR master mix and extracted samples into the amplification plate after sample extraction on the VERSANT kPCR Sample Prep instrument SP. The new PCR setup software automates this process. Performance of VERSANT HCV Genotype 2.0 Assay (LiPA) using manual PCR plate setup vs. automated PCR plate setup was evaluated. Two unique HCV subtype 1a donor sera, initially genotyped using an NS5B sequencing method, were used to prepare replicate samples at three different viral concentration levels: 600 IU/mL (1.5 × LoD), 1×10^4 IU/mL, and 6×10^5 IU/mL. The study design included a total of 368 HCV subtype 1a panel members for each method. The genotyping rate (GR) and genotyping accuracy (GA) were calculated to compare the manual and automated PCR setup methods. **Results:** All runs in the study were valid, and all 368 samples tested for each method produced interpretable results. The GR was 100% for all viral concentration levels with both automated and manual PCR plate setup, and all valid interpretable samples from each plate setup method were accurately genotyped (100% GA). The lower limit of the confidence interval (Wilson Score Method) was 95.99% for samples with n = 92 (6×10^5 and 10^4 IU/mL) and 97.95% for samples with n = 184 (600 IU/mL). **Conclusion:** The present study demonstrates no difference in genotyping rate and accuracy between manual and automated PCR setup on the VERSANT kPCR Sample Prep instrument. The VERSANT HCV Genotype 2.0 Assay (LiPA) continually provides accurate identification of HCV genotypes as shown by GR and GA rates of 100%, while providing high throughput and automated workflow. *The VERSANT HCV Genotype 2.0 Assay (LiPA) (Reagents: Genotype 2.0 Kit, Amplification 2.0 Kit, and Control 2.0 Kit) is CE-marked in Europe and FDA-approved in the U.S. HOOD05162002791113

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Evaluation of VACUETTE® BCA FastSeparator Blood Collection Tube for Routine Chemistry Assays

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Background: Clotting time is an important factor in the work flow of blood collection tubes for routine chemistry testing from serum. To optimize laboratory workflow, reduced turnaround times are expected to provide precise test results. The key target of VACUETTE BCA Fast tubes consists in the faster processing time from blood collection to result availability. The faster clotting time in the VACUETTE BCA Fast tube provides a clotted sample by the time the sample reaches the laboratory and allows for immediate testing. Tubes containing a gel separator offer the option of replicate measurements up to 48h at refrigerator temperature. **Methods:** This study was conducted in order to demonstrate the performance of VACUETTE BCA Fast tubes for routine chemistry analysis up to 48h in comparison to VACUETTE Serum Separator tubes. Venous blood was drawn from 50 healthy donors into two tubes. Tubes without thrombin were centrifuged after 30 min clotting time, and the tubes with thrombin were centrifuged after 5 min clotting time. All samples were centrifuged for 10 min at 1800g. Initial values of routine chemistry assays were determined on an AU680 and DxI800 from Beckman Coulter (Beckman Coulter, precision within-run <3%, total <3%). All samples were stored in an upright position at 4-8°C for replicate testing after 24h and 48h. Comparison analysis was performed at all time points. Clinical evaluation was based on CLIA (Allowable Total Error Table by Data Innovations). **Results:** Equivalency for VACUETTE BCA Fast tubes to VACUETTE Serum Separator tubes was shown for routine chemistry assays on Beckman Coulter for healthy donors. Provided a completely clotted sample and clear serum specimens, no significant deviations were found for initial values as well as at 48h for 37 biochemical assays tested in both tubes according to CLIA tolerances. In agreement to literature, slight systematical deviations in the thrombin tubes were found for some assays such as sodium, potassium, chloride, and glucose due to the faster coagulation process. Stability over 48h was shown for all assays except troponin I (6h). **Conclusion:** The thrombin tube gave comparable test results to current serum separator tubes for most common biochemical assays in clinical laboratories. The blood collection tube containing thrombin provides rapid turnaround times in the laboratory by shortening the clotting time, providing accurate testing results and being suitable for emergency testing, however, those tubes are not recommended for patients on heparin therapy, thrombin inhibitor therapy or with deficiency in the clotting factors.

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Evaluation of IgG Index as a Biomarker for Multiple Sclerosis: Experience from a Tertiary Care Center in Saudi Arabia

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Background: The diagnosis of multiple sclerosis (MS) is based on neurologic history, clinical findings on examination and exclusion of other disorders. The single most consistent laboratory abnormality in patients with MS exclusive of magnetic resonance imaging is increased oligoclonal immunoglobulin in cerebrospinal fluid (CSF). The presence of oligoclonal bands (OCB) in CSF but not in the serum is a strong indication of intrathecal antibody synthesis and is found in nearly all patients with MS. Furthermore, CSF IgG index is used as a measure of IgG production within the CNS; however, its biomarker potential for MS is not firmly established. **Aim**: To determine the correlation between OCB and IgG index and assess the specificity of IgG index for the diagnosis of MS.

Methods: This study was conducted on 209 patients (92 males and 117 females) with suspected CNS immune mediated disorders (CIMD) or multiple sclerosis (MS). Sera and CSF samples were analyzed for IgG and albumin for computing IgG index. (Siemens BN II) Isoelectric focusing-immunoblotting was used for the detection of OCB. (H Sebia Hydrasys 2 System) Presence of three or more bands in CSF but their absence in serum indicated a positive pattern. Data were analyzed by one-way analysis of variance followed by Dunnett's test for comparing means and Spearman's test for correlations. **Results:** The positive OCB were observed in the CSF of 76 (36%) patients. The OCB were missing in the CSF of 113 patients while 20 patients showed matching OCB (both in CSF and sera). IGG index was significantly higher in OCB positive patients (1.35 ± 0.17) as compared to OCB negative (0.64 ± 0.07) or OCB matching patients (0.51 ± 0.02) (ANOVA F=11.17, P<0.001). There was a significant correlation between OCB and IgG index (R^2 = 0.464, P<0.001). Using a previously reported IgG index cut-point of 0.85, only few predictions were false-positive (2.25%) whereas the false-negativity was extremely high (50%). **Conclusions:** IgG index lacks the specificity for the diagnosis of MS due to extremely high false-negative predictions. Utilizing radiological examination in addition to OCB are required for more authentic validation of IgG index.

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Clinical performance of Dynamiker® Cryptococcal Antigen Lateral Flow Assay compared to IMMY® CrAg LFA in diagnosis of Cryptococcus

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Background
Cryptococcus is one of the major invasive fungal disease and early diagnosis plays an important role in treatment. Detection of cryptococcal antigen in blood and cerebrospinal fluid (CSF) is critical mycological evidence in diagnosis of Cryptococcus. In this study we evaluate the clinical performance of newly developed Cryptococcal Antigen Lateral Flow Assay(LFA) by comparing with IMMY® CrAg LFA.
Material and Method
Total of 82 CSF samples and 94 serum samples from patients who were at risk of Cryptococcus were retrospectively collected. Serum or CSF Cryptococcal Antigen levels were determined by using Dynamiker® Cryptococcal Antigen LFA and IMMY® CrAg LFA at the same time. An additional test was performed if initial test result was positive.
Results
Twenty-six of 82 CSF samples were determined as positive by Dynamiker® LFA while 25 samples were positive in IMMY® CrAg LFA. There were 37 positive serum samples determined by Dynamiker® LFA compared with 35 positive samples from IMMY® CrAg LFA. All IMMY® CrAg LFA positive samples were also Dynamiker® LFA positive in this study. The Kappa value for CSF samples and serum samples were 0.972 and 0.955, respectively.
Conclusion
The clinical performance of Dynamiker® Cryptococcal Antigen LFA is highly consistent with that of IMMY® CrAg LFA
Table1 Comparison of CSF and Serum samples

CSF sample	IMMY LFA			
	Positive	Negative	Total	
DNK LFA	Positive	25	1	26
	Negative	0	56	56
	Total	25	57	82
Serum sample	IMMY LFA			
	Positive	Negative	Total	
DNK LFA	Positive	35	2	37
	Negative	0	57	57
	Total	35	59	94

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Evaluation of biochemical biomarkers in the CSF and with genotyping for the risk of Alzheimer's disease in patients with some neurological impairment.

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Background: The increase in life expectancy and the increasing rate of elderly people leads to a population projection for 2050 of 115400000 elderly with Alzheimer's disease (AD) in the world. The literature shows that the pathological process of AD begins at least 10-20 years before the onset of the first symptoms of dementia. Therefore, the prediction and treatment of asymptomatic individuals, in whom degeneration is not yet severe, is crucial. Several studies with AD have been carried out by genotyping for apoprotein E (ApoE) and its alleles (E2, E3 and E4) as well as the main biochemical markers in the cerebrospinal fluid (CSF) that relate to this disease as: total tau protein (t- tau), Beta Amyloid- 42 (Aβ-42), which is identified early, before the onset of the first symptoms of AD. Thus, measurement of Aβ-42 in CSF may facilitate the diagnosis of incipient AD in patients with mild cognitive impairment. Recent studies have evaluated the diagnostic utility of the Aβ-42 / Aβ-40 ratio, although Aβ-40 is slightly increased or unchanged in the CSF of patients with AD. The t-tau is the major component of neurofibrillary tangles which is another key neuropathological feature of AD. It is known that these parameters also change in many conditions and neurological diseases and can also lead to dementias reaching to AD. In this study, the authors studied two groups of patients with neurological impairment of diabetic etiologies: infectious and non-infectious (other causes), evaluating biochemical parameters and genotyping for AD risk.METHODS: 44 patients who had been prescribed CSF for some diagnosis or monitoring hypothesis were invited to participate in this study and signed the TCLE. We divided the clinical groups into two: patients with suspected neurological involvement due to infectious causes (n = 26) and patients with noninfectious involvement. The following parameters were determined: (Aβ-42 and Aβ-40) and (t-tau) by the Elisa / Euroimmun Medizinische Labordiagnostika AG methodology. Apo E genotyping was determined by salivary collection by PCR method, using primers specific for the detection of E2, E3 and E4 alleles of ApoE gene polymorphism.Results:The results showed that, using ANOVA, that the Apo E genotype and the t-tau variation did not present a significant difference (p = 0.10) for all groups. Aβ-42 presented a difference between the E3 / E3 group, which showed higher levels than the E3 / E4 group (p = 0.006). Likewise, the Aβ-42 / Aβ-40 ratio presented a significant difference between the E3 / E3 group compared to the E3 / E4 group. Regarding clinical indication, the E3 / E4 group with infectious neurological involvement had a higher Aβ-42 / Aβ-40 ratio than the non-infectious E3 / E4 group. Patients with E2 / E3 from the infectious group showed a lower ratio than the E2 / E3 group from the non-infectious group.Conclusion: in conclusion of the findings, the noninfectious E2 / E3 group presented a lower risk for AD when compared to the infectious group. The E3 / E3 infectious group presented a higher risk than both E3 / E4 groups.

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Evaluation of C-reactive protein and white blood cell count as an early infection marker after the surgical operation of hip fracture

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Background: C-reactive protein (CRP) is a known inflammatory and infection marker. We examined the serial serum levels of CRP and white blood cell (WBC) count after the operation of hip fracture to evaluate the usefulness of CRP and WBC count as an early infection marker after the surgical operation of hip fracture. Methods: Postoperative CRP level and WBC count in 279 patients with hip fracture from January 2013 to December 2016 were retrospectively examined. The patients had the serial test results of both markers for more than 7 days after the operation. Mean and SD of both markers were obtained according to the day after operation Results: Two hundred seventy-three of 279 (97.8%) had no infection after the surgical operation and the other 6 (2.2%) had infection. Postoperative mean WBC count of the patients without infection was follows: 10.8 K/uL just after the operation, 9.24 K/uL at first day, and 8.81 K/uL at second day. Postoperative mean WBC count of the patients with infection was 14.8 K/uL, 14.4 K/uL, and 15.3 K/uL, respectively. There was a statistical difference in WBC count between both

patient groups. Postoperative mean CRP of the patients without infection was follows: 5.65 mg/dL just after the operation, 8.36 mg/dL at first day, 11.0 mg/dL at second day, 9.15mg/dL at third day, 7.04 mg/dL at fourth day, 4.59 mg/dL at fifth day, 4.22 mg/dL at sixth day, and 3.44 mg/dL at seventh day. The CRP level reached the maximum value on the second day in the patient without infection. Postoperative mean CRP of the patients with infection was follows: 10.29 mg/dL just after the operation, 16.22 mg/dL at first day, 8.71 mg/dL at second day, and 16.43 mg/dL at third day. There was a statistical difference in CRP between both patient groups. Conclusion: CRP and WBC count could be an early infection marker after the surgical operation in the patients with femoral fracture.

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Effects of δ-Tocotrienol on Glycemic Control and Inflammatory Biomarkers in the Diabetic Patients

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Background: Diabetes mellitus is a complex, long standing health problem especially in Asian countries. Inflammation, increased oxidative stress and impaired insulin action are the main factors for development of multiple diabetes associated micro and macro vascular complications. Being potent anti-oxidative and anti-inflammatory agent, tocotrienol is continuously re-evaluated for a long time in different chronic diseases. Due to the diverse results and limited number of studies in the diabetic patients, there is need of further clinical trials in order to determine the impact of purified delta tocotrienol in type 2 diabetes mellitus patients. **Objective**: To find out the effect of delta tocotrienol (250 mg) supplementation along with recommended diabetic medications on fasting glucose, glycated Hb, total cholesterol, triglycerides and hs C-reactive protein (hs-CRP) in patients of type 2 diabetes mellitus. **Method**: In this randomized control trial, 54 subjects age ≥30 years with serum fasting glucose and HbA1c levels ≥ 7 mmol/L and HbA1C ≥ 6.5% were included. Persons having history of acute illness, liver, renal, thyroid disorders or malignancy and history of taking anti-inflammatory drugs, vitamin E were excluded from study. Patients were randomized into two groups, 27 patients in group A and 27 in group B by a simple random draw. Subjects in the group A were given capsules containing 90% pure δ-tocotrienol 125mg twice daily and group B was provided placebo twice daily for three months. Total 5 ml blood was collected for analysis of biochemical markers at the start of study and after three months. To compare the baseline and 3 month values in both groups paired student t-test was used. Statistical significance was set at $p < 0.05$. **Results:** Pre vs post levels of diabetic associated biomarkers including fasting glucose, HbA1c, total cholesterol, triglyceride and hs-CRP in tocotrienol group were 14.10±3.15 vs 11.65±2.71 mmol/L, 10.07±2.0 vs 8.98±1.70 %, 5.01±1.21 vs 4.47±1.33 mmol/L, 2.61±2.42 vs 2.40±1.25 mmol/L and 4.76±4.41 vs 2.95±2.73 mg/L were significantly decreased ($P<0.05$) respectively. In Placebo group, pre vs post levels of these variables were 14.56±3.24 vs 14.12±3.01 mmol/L, 10.77±2.11 vs 10.45±1.83 %, 4.59±1.50 vs 4.53±1.31 mmol/L, 2.28±1.54 vs 2.22±1.15 mmol/L, 4.99±4.43 vs 4.78±3.65 mg/L (p=NS) respectively. **Conclusion**: The consumption of delta tocotrienol supplementation in addition to antidiabetic drugs at early phases of the disease can be helpful in the prevention of long term complications by improving glycemic control and reducing inflammatory process in the diabetic patients. Delta-Tocotrienol demonstrated significant reduction in serum lipid parameters which are associated with cardiovascular diseases in the diabetic patients.

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CoQ10 and Total antioxidant capacity in early breast cancer

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Background: Oxidative stress in the body, whether resulting from endogenous or exogenous factors, has been associated with the development of breast cancer. We aimed to assess Coenzyme Q10 (CoQ10) and Total Antioxidant Capacity (TAC) as indicators of antioxidant state in women with early breast cancer. Method: Serum samples were collected from 80 recently diagnosed non-intervened female breast cancer patients and 20 healthy control female volunteers. TAC was measured spectrophotometrically. CoQ10 was measured using HPLC technique using HPLC ClinRep™ kit from Recipe, Germany (Code: 31000) using a C18 column and an isocratic mobile phase. Results: A statistically significant increase in TAC levels was found in breast cancer patients (1.80 ± 0.35 mMol/L) when compared to the control group (1.62 ± 0.29 mMol/L). Women having TAC levels above 1.5 mMol/L were 2.2 times more at risk of developing breast cancer than those with values below 1.5 mMol/L with odds ratio 2.211 (1.012-65.526) with a 95% confidence interval and p value of 0.049. Multiple linear regression analysis performed nullified the effect of BMI (bdy mass index) and found TAC to be an independent predictor of breast cancer. The adjusted odds ratio has also found TAC to be statistically significant. It was found to be 5.944 (1.203-29.371) with a p value of 0.029 at a 95% confidence interval. CoQ10 levels were significantly higher in the breast cancer group (1337.67 (630.36-3333.51) µg/L) when compared to the control group (1195.30 (647.58–1775.01) µg/L). A CoQ10 level above 1600 µg/L was found to have an odds ratio of 7.878 (0.976-63.260) with a 95% confidence interval which possessed a risk for breast cancer development yet this risk was found to be of borderline significance (p= 0.053). Conclusion: significantly higher TAC and slightly higher CoQ10 levels in the breast cancer group were found compared to the control group. It is still unclear whether the increased host antioxidant defenses offer a selective growth advantage to tumor cells over their surrounding normal cells or serve as a protective measure by the body in an attempt to correct the assault triggered by the ROS. We suggest a potential role for antioxidants & CoQ10 in the development and progression of breast cancer. However larger sample size is recommended. Circulating CoQ10 may not be indicative of intracellular CoQ10 yet may be a response to chronic inflammation, heightened systemic or tissue-specific oxidation.

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A not so unusual complication? A case of urinary calculus formation caused by sulfamethoxazole-trimethoprim therapy

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Background: Sulfamethoxazole-trimethoprim (TMP-SMX) is a commonly employed antibiotic used for mild-to-moderate bacterial infections and as prophylaxis against opportunistic infections. Genitourinary side-effects such as crystalluria and urolithiasis have been reported, but infrequently. Furthermore, medication package inserts for common commercial preparations available in North America do not list urinary calculi formation as a potential adverse reaction or side-effect. Therefore, this potential complication may remain largely unrecognized by laboratories and clinicians. We describe a case of a bladder (vesical) calculus comprised of the TMP-SMX metabolite, N4-acetylsulfamethoxazole, as well as the validation of this metabolite in a basic urinary calculi spectral library. **Methods:** The urinary calculus was received by a regional reference laboratory for composition analysis by Fourier-transform infrared (FT-IR) spectroscopy. Observations were made regarding physical characteristics (size, colour, shape, and consistency). The specimen was ground, mixed with potassium bromide and analyzed by a Nicolet iS10 FT-IR spectrometer with OMNIC Specta software. Spectral scans were compared to a basic urinary calculi spectra library as well as a pure chemical spectra library. **Results:** The calculus was retrieved from the bladder of a 66-year old male recovering from radical prostatectomy with no prior history of urinary calculi. Post-prostatectomy, the patient required prolonged catheterization during which he experienced difficulties with a blocked catheter. During that time period, he received Septra DS for two weeks. When assessed for catheter removal, a calculus was observed at the bladder neck, next to the catheter. This stone was mechanically broken and submitted for laboratory analysis. The stone was tan in colour, 4x3x1 mm in size with irregular shape, and a hard consistency. Initial analysis by FT-IR spectroscopy against a basic urinary calculi library provided no spectral match. However, comparison to a pure chemical spectra library provided a >95% probability match to N4-acetylsulfamethoxazole. To verify the composition match, a portion of the stone was sent for confirmation testing by a second reference laboratory, which confirmed the sulfa-drug metabolite. After stone removal, the patient was kept off Septra DS and has had no evidence of stone re-occurrence. **Conclusion:** Precipitation of sulfonamides may occur more frequently than previously recognized. A prior case of N4-acetylsulfamethoxazole urinary calculi formation noted that stone formation coincided with an obstructing calcium oxalate stone producing oliguria and acute azotemia (J. Urol. 1977; 117:397). We report an additional case of TMP-SMX-mediated stone formation, concurring with a clinical history of oliguria. Since sulfa-drug metabolites may not be included in basic urinary calculi spectra libraries, laboratories providing urinary calculi analysis should ensure that their system detects and classifies such stones. Clinicians should be aware that sulfa-drug metabolite stones may form with TMP-SMX treatment, particularly in patients with reduced urinary flow. Medication package inserts for TMP-SMX should be updated to include urolithiasis as a potential side-effect.

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Prevalence and related factors to dyslipidemias in university students

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Background: The dyslipidemias are a set of pathologies characterized by alteration of one and/or more parameters of the lipid profile; dyslipidemia is the biochemical manifestation of genetic variations or secondary to lifestyle factors; it also constitutes an important modifiable risk factor due to its direct relationship with the coronary disease. The objective of the study was to determine the prevalence and factors related to dyslipidemias in university students **Methods:** Descriptive, cross-sectional, prospective study that was carried out during November 2012, in the study, 220 students were included through a non-probabilistic convenience sampling. Lipid profile measurements were made by enzymatic method and precipitation for cholesterol fractions, as well as anthropometric measurements; in addition, data such as age, sex, and personal and family history were recorded. The identification of dyslipidemias was based on the results of the lipid profile according to what was established by the NCEP - ATP III. A database was created in Excel 2010; continous data were expressed descriptive statistic and frequency/percentages were qualitative variables. Multiple analysis a generalized linear model was used, family binomial link log to identify factors related to dyslipidemia. The software STATA version 13 was used **Results:** A total of 220 students who participated in the study were evaluated, 167 (75.91%) were female, the average age was 21.19 years. The 80 (54.30%) and 38 (22.75%) of women presented central obesity and overweight respectively.The prevalence of dyslipidemia was 134 (60.91%). HDL was found to be reduced in 117 (53.18%) and 8 (3.64%) had alterations in all parameters of the lipid profile. No relationship was found between dyslipidemia and body mass index, family and personal history. In the multiple analysis, the prevalence of having dyslipidemia was associated with central obesity (PR 1.62; IC95% 1.30 - 2.0, p <0.05) and the male sex (PR 0.61; IC95% 0.44 - 0.86, p<0.05), after adjusting for family and personal background. **Conclusion:** It is concluded that there is a high prevalence of dyslipidemia (60.91%), with low HDL levels being one of the most frequent parameters; furthermore the main factors related to dyslipidemia were central obesity and the university students' sex.

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The Hepcidin in the non-alcoholic fatty liver disease

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Background: Hepcidin is a peptide mainly produced by hepatocytes and, through a connection with ferroportin, it regulates iron absorption in the duodenum and its release of stock cells. The non-alcoholic fatty liver disease (NAFLD) is associated with resistance to insulin action, metabolic syndrome and hyperferritinemia. The mechanism of increased iron absorption in NAFLD is incompletely understood but is likely caused by decreased hepcidin production in the diseased liver. **Objective:** This study evaluates hepcidin levels in patients with NAFLD and its relationship with iron overload and severity of liver disease**. Methods:** Patients with diagnosis of NAFLD after hepatic biopsy and clinical cirrhosis were included in this study. We exclude alcohol consumption above 20g / day, drugs that cause liver damage and infectious or autoimmune hepatitis. Hepcidin was measured by immunoassay DRG 25 Bioactive ELISA-Etimax, normal values:0,91-33,55ng/mL and Ferritin for ICMA (Beckman Coulter).The correlations between hepcidin, ferritin and histopathological findings after hepatic biopsy were calculated by correlation coefficient (pearson). Patients were divided as to severity of liver disease in hepatic steatosis and cirrhosis (Metavir-Fibrosis F = 4). A total of 48 patients performed magnetic resonance (MR) for quantification of the iron hepatic deposit. **Results:** Eighty-six patients were studied, the majority of females (72%), with a mean age of 56 years ± 10, BMI 33 ± 6 kg / m2, CA 110 ± 12 cm). Of these, 64% had Diabetes, 81% hypertension and 97% metabolic syndrome. Of the 73 biopsy patients 31% had mild steatosis, 48% moderate and 21% severe. And Of this total of 73 patients 66% had Steatohepatitis and 21% had cirrhosis; Ferritin was elevated in 28% (21% with increase of 1 to 2 times the normal and 7% with an increase of more than twice the normal value. Only 8% had iron overload in MR, all cases with mild overload. We found a negative correlation between hepcidin and ferritin(r = 0.356 p = 0.002) and also between hepcidin and liver disease severity, with lower levels of hepcidin in patients with hepatic cirrhosis (46 ± 22 vs. 29 ± 20; p = 0.005). **Conclusion:** Patients with advanced DHFBD (cirrhosis) have lower levels of hepcidin; however, despite their correlation with serum ferritin, There was no correlation between Hepcidin and a significant iron overload in the liver observed in MR.

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A comparative study to assess serum sFlt-1 to PlGF ratio in pregnant women with and without Preeclampsia

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Background
Preeclampsia is a disorder of widespread vascular endothelial malfunction that occurs after 20 weeks of gestation. Abnormalities in the development of placental vasculature early in pregnancy may result in relative placental underperfusion, which then leads to release of antiangiogenic factors into the maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations. Imbalance in, placental soluble Fms like tyrosine kinase -1 (sFlt-1) which is antiangiogenic factor; and placental growth factor(PlGF) which is involved in angiogenesis during placenta and fetus development, is proved to have role in endothelial damage in Preeclampsia. At a time when most public health facilities are lacking standardized testing tools for pre-eclampsia and eclampsia, there is need of an innovate and improved tool for screening of preeclampsia, which is the leading cause of maternal mortality in Nepal. This study was designed to compare sFLT1: PLGF ratio in pregnant women with and without Preeclampsia attending Tribhuvan University Teaching Hospital (TUTH). Similarly, correlation of sFlt1: PlGF ratio with diastolic blood pressure and severity of proteinuria in women with preeclampsia was also done.
Method
A case control study was done in Gynecology and Obstetrics department of TUTH involving forty-four subjects with preeclampsia and forty-four age and gestational weeks matched, normal pregnancy as controls. Cases were divided into mild and severe group of preeclampsia according to the criteria defined by the American College of Obstetricians and Gynecologists. Blood pressure, urinary protein, serum sFlt-1, serum PlGF and sFlt-1: PlGF ratio were compared in both case and control. Concentration of sFlt-1 and PlGF were measured with commercially available ELISA kits. SPSS ver. 17.0 was used to analyze the data. Tests were performed with T test, Mann–Whitney test, and Spearman's rank correlation test. Normally distributed variables were expressed in terms of mean ± SD. A p-value <0.05 was considered statistically significant.
Results
There was no significant difference in age and period of gestation in both study groups. Mean concentration of sFlt-1 in preeclampsia was higher (2575.50 ±775.03 pg/mL) compared with normal pregnancy (453.75 ± 156.24 pg/mL). Mean concentration of PlGF was lower in preeclampsia (86.31 ± 26.9 pg/mL) compared with normal pregnancy (155.41 ± 63.89 pg/mL). Ratio of sFlt-1 and PlGF concentration was significantly higher in preeclampsia (P value 0.000) than in normal pregnancy. Similarly, the diastolic blood pressure significantly correlated with the sFlt-1: PlGF ratio in preeclamptic group (p-value 0.000) whereas the severity of proteinuria did not significantly correlate with the ratio of sFlt-1: PlGF in preeclamptic women (p-value 0.773).
Conclusion
sFlt-1 level is increased and PlGF level is decreased in preeclampsia compared to the normal pregnant women. sFlt-1/PlGF ratio is significantly higher in women with preeclampsia than in normal control. This ratio can be a potential marker for diagnosis of preeclampsia.

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Post-Partum Glucose Testing: Missed Opportunities for Assessing and Preventing Diabetes mellitus in women with Glucose Intolerance in Pregnancy

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Background
Gestational diabetes mellitus (GDM) is a common medical problem in pregnancy and predict future Type 2 Diabetes Mellitus (T2DM). Expert guidelines encourage postpartum glucose testing at the 6 weeks post-natal visit to properly evaluate and manage women who were diagnosed with GDM during pregnancy. We followed up women diagnosed with GDM and overt DM to examine the post-natal care, prevalence of postpartum glucose testing and the factors associated with testing.
Methods
This was a retrospective study of 142 women who were identified as having GDM or Overt DM using the Modified WHO 2013 diagnostic criteria after a 75-g oral glucose tolerance test (OGTT) between April 2013 and April 2017 among preg-

nant women referred to the metabolic Clinic of the Clinical Chemistry Department of Jos University Teaching Hospital. Fifty eight women responded to followed up phone calls to determine their post- pregnancy status. Socio-demographic data and obstetric history were obtained from hospital and laboratory records.
Result
The Mean (SD) age of the women was 33.6(5.0) yrs; 34(58.6%) and 24 (41.4%) had GDM and overt DM respectively. Most of the women had tertiary education 47(81%); were less than 35 years of age 34(58.6%) and Grandmultiparous 39(67.2%). Median (IQR) Gestational Age at testing was 30 (23.5-32.0) weeks. Only twenty women (34.5%); 4(6.9%) by OGTT and 16 (27.6%) by random glucose were tested six weeks post delivery. Thirty seven (63.8%) and 19 (32.8%) had fasting and random glucose testing respectively while 8(13.8%) did not have any form of glucose testing after delivery. Most of the testing were done by Point of Care Testing (POCT) 42(72.4%); 10 (17.2%) were tested in a clinical laboratory. Only 28 (48.3%) had been counseled to repeat OGTT post-delivery while 19 (32.8%) were referred for further treatment. A diagnosis of overt DM was significantly associated with random glucose testing after 6 weeks visit (P= 0.019) and testing carried out in the laboratory P=0.043. The category of diagnosis (GDM or Overt DM) was not associated with repeat OGTT/random glucose, testing by POCT, counseling for repeat OGTT or referral for further treatment (P> 0.05).
Conclusion
This study highlights that OGTT or random glucose test at 6 weeks post delivery for women diagnosed with GDM or overt DM is very low. Poor counseling and referral for treatment suggest a gap in post-partum care given to women with glucose intolerance in Pregnancy and represents missed opportunities for assessing and preventing Diabetes mellitus and Cardiovascular Diseases in such women. In view of the increased risk of T2DM in women diagnosed with GDM, there is urgent need for local guidelines and coordinated multidisciplinary approach to follow-up testing for such women. Post-partum screening for DM and CVD risk assessment should be incorporated into existing integrated care programmes for mother and child at the 6-weeks post-partum visit. Laboratories should play more prominent role in post-partum glucose testing and closer collaborations between clinical laboratories and clinicians is crucial for slowing the progression to overt DM and attendant complications.

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Clinicopathological features and survival outcome according to KRAS, NRAS and BRAF mutation status in patients with colorectal cancer

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Background: Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide. One of the fundamental processes driving the initiation and progression of CRC is the accumulation of a variety of genetic and epigenetic changes in colonic epithelial cells. In this retrospective observational study, frequencies and clinicopathological features of KRAS, NRAS, BRAF mutations were evaluated in patients with colorectal cancer. Among patients who treated, progression-free survival (PFS) and overall survival (OS) were appraised according to gene status. **Methods:** Between 2002 and 2017, a total of 246 patients with colorectal cancer who were treated and followed up in our oncology center were analyzed. KRAS, NRAS, BRAF mutations analysis was performed using quantitative PCR evaluation of the DNA from the tumor tissues. Progression-free survival (PFS) and overall survival (OS) were calculated for each of the patients and the relationship between survival and mutation status was evaluated. **Results:** One hundred and fifty four (62.6 %) were male and ninety two (37.4 %) were female, with a median age of 55 years (range 23-86). Based on tumor localization, 153 patients (62.2 %) were classified as colon cancer patients and 93 patients (37.8 %) were classified as rectal cancer patients. The majority of patients (86.2 %) had adenocarcinoma histology, while 24 cases (% 9.8) had mucinous adenocarcinoma. Among 246 patients, mutations in KRAS exon 2, exons 3 or 4, NRAS and BRAF were detected in 33.5 %, 1.6 %, 1.2 %, 4.0 % and 1.6 %, respectively KRAS mutations were detected in 103 of the patients (41.9 %). The median overall survival (OS) and progression-free survival (PFS) time were 39.9 and 7.5 months for the patients with KRAS mutations tumors. For the patients with all wild-type tumors, OS and PFS were 43.4 and 13.3 months. **Conclusion:** Our data suggest that mutations in KRAS are associated with inferior PFS and OS of CRC patients compared with patients with non-mutated tumors.

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Multicenter Evaluation of Ceftazidime/Avibactam MIC Results for Enterobacteriaceae and Pseudomonas aeruginosa Using MicroScan Dried Gram Negative MIC Panels

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Background: A multicenter study was performed to evaluate the accuracy of ceftazidime/avibactam on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels. **Materials/Methods:** For efficacy, an evaluation was conducted at three sites by comparing MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 618 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested using turbidity and Prompt™* methods of inoculation. For challenge, a set of 116 organisms was tested on MSDGN panels at one site. For reproducibility, a subset of 16 organisms was tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels, prepared according to CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2°C and read visually at 16-18 hours. FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* and *Pseudomonas aeruginosa* ≤ 8/4 S and ≥ 16/4 R. **Results:** When compared to frozen reference panel results, essential and categorical agreements for all isolates tested in Efficacy and Challenge are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		VMJ^ %		MAJ^ %	
	T	P	T	P	T	P	T	P
Visually	98.8 (725/734)	97.7 (717/734)	99.2 (728/734)	99.0 (727/734)	6.5 (2/31)	3.2 (1/31)	0.1 (1/703)	0.3 (2/703)
Walk Away	98.9 (726/734)	95.2 (699/734)	98.8 (725/734)	98.4 (722/734)	3.2 (1/31)	3.2 (1/31)	0.3 (2/703)	1.0 (7/703)
auto SCAN-4	98.8 (725/734)	97.3 (714/734)	99.2 (728/734)	98.9 (726/734)	6.5 (2/31)	6.5 (2/31)	0.0 (0/703)	0.0 (0/703)

T = Turbidity inoculation method, P = Prompt* inoculation method
^ = calculated without 1 well dilution errors

Reproducibility among the three sites were greater than 95% for all read methods for both the turbidity and Prompt* inoculation methods. **Conclusion:** This multicenter study showed that ceftazidime/avibactam MIC results for *Enterobacteriaceae* and *Pseudomonas aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels.
* PROMPT is a registered trademark of 3M.

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Multicenter Evaluation of Ceftolozane/Tazobactam MIC Results for Enterobacteriaceae and Pseudomonas aeruginosa Using MicroScan Dried Gram Negative MIC Panels

R. K. Brookman[1], A. Harrington[2], J. A. Hindler[3], M. Traczewski[4], S. Campeau[3], S. DesJarlais[2], D. Beasley[4], C. J. Hastey[1], D. Roe-Carpenter[1].
[1]Beckman Coulter, Inc., West Sacramento, CA, [2]Loyola University Medical Center, Maywood, IL, [3]UCLA Health System, Los Angeles, CA, [4]Clinical Microbiology Institute, Wilsonville, OR

Background: A multicenter study was performed to evaluate the accuracy of ceftolozane/tazobactam on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels. **Materials/Methods:** For efficacy, an evaluation was conducted at three sites comparing MICs obtained using the MSDGN to MICs using a CLSI broth microdilution reference panel. A total of 575 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested using turbidity and Prompt™* methods of inoculation. For challenge, a set of 118 organisms was tested on MSDGN panels at one site. For reproduc-

ibility, a set of 17 organisms was tested on MSDGN panels at three sites. MSDGN panels were incubated at 35 ± 2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels, prepared according to CLSI methodology, were inoculated using the turbidity inoculation method. Frozen reference panels were incubated at 35 ± 2°C and read visually at 16-18 hours. FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 2/4 S, 4/4 I, and ≥ 8/4 R and *Pseudomonas aeruginosa* ≤ 4/4 S, 8/4 I, and ≥ 16/4 R. **Results:** When compared to frozen reference panel results, essential and categorical agreements for all isolates tested in Efficacy and Challenge are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		VMJ %		MAJ %		MIN %	
	T	P	T	P	T	P	T	P	T	P
Visually	96.5 (669/693)	95.2 (660/693)	98.0 (679/693)	97.0 (672/693)	0.0 (0/63)	1.6 (1/63)	1.0 (6/623)	1.0 (6/623)	1.2 (8/693)	2.0 (14/693)
Walk Away	96.4 (668/693)	92.8 (643/693)	97.8 (678/693)	95.2 (660/693)	1.6 (1/63)	3.2 (2/63)	0.3 (2/623)	3.0 (19/623)	1.7 (12/693)	1.7 (12/693)
auto SCAN-4	95.5 (662/693)	94.9 (658/693)	98.1 (680/693)	97.1 (673/693)	1.6 (1/63)	3.2 (2/63)	0.5 (3/623)	1.1 (7/623)	1.3 (9/693)	1.6 (11/693)
T = Turbidity inoculation method, P = Prompt* inoculation method										

Reproducibility among the three sites were greater than 95% for all read methods for both turbidity and Prompt* inoculation methods. **Conclusion:** This multicenter study showed ceftolozane/tazobactam MIC results for *Enterobacteriaceae* and *Pseudomonas aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels. * PROMPT is a registered trademark of 3M.

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Prevalence of MGUS and risk factor evaluation for progression to malignancy in a mexican population

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Background: Monoclonal gammopathy of undetermined significance (MGUS) is characterized by the presence of a monoclonal protein in the serum of asymptomatic individuals who do not meet the diagnostic criteria for other plasma cell disorders. It is defined as having <30g/L of a serum monoclonal protein, clonal bone marrow plasma cells <10% and the absence of end-organ damage that can be attributed to the plasma cell disorder. MGUS is present in approximately 3% of individuals aged 50 or older and increases with age. Whilst most MGUS patients have a stable condition and remain asymptomatic, a small proportion will progress to MM or a related B-cell or lymphoid cancer. This equates to a 1%-per-year lifelong risk of malignant transformation. The actual prevalence in Mexican population is unknown. **Objective:** To determine the prevalence of MGUS in the Yucatan population in Mexico and risk stratify when possible. **Methods:** We studied Yucatan native patients older than 30 years old from January 2015 until June 2017. An SPE (Interlab) was performed to detect M protein. All positive SPEs (monoclonal bands) samples were tested by IFE (Interlab) and Free Light Chains (FLC, Freelite, The Binding Site) according to manufacturer's instructions and using the suggested reference range for FLC ratio (0.26-1.65). **Results:** We analyzed 2053 serum samples (1020 men and 1033 women) and detected 61 cases of MGUS among them. The general prevalence rate was 2.97% in our population. 64% of them had an IgG M protein, 15% had an IgA M protein, 13% has an IgM M protein and we found an 8% of MGUS with only FLC as M protein. Thirty seven patients were further analyzed for risk stratification and FLC ratio analysis showed that 57% of samples had an abnormal FLC ratio. When all risk factors were analyzed (level of M protein ≥ 15g/L, M protein IgA or IgM and abnormal FLC ratio) we found that 24% of MGUS patients had no risk factors, 46% had 1 risk factor, 27% showed 2 risk factors and 3% had the 3 risk factors. The most repetitive risk factor in our study population was an abnormal FLC ratio, in 21 patients. **Conclusion:** For the first time, we were able to analyze MGUS patients in a Yucatan population, where the prevalence was found to be 2.97% which is different from general Mexican population according to the literature (Agarwal *et al*. Clin Cancer Res 2013). In our cohort, most MGUS patients showed to have 2 MM risk factors and 57% of Yucatan MGUS patients have abnormal FLC ratios, which is notably higher than the 33% reported for the general population (Rajkumar *et al*, Blood 2008). Whilst the 1% average annual risk of MGUS developing into MM or a related condition is well documented, progression among individual MGUS patients is highly variable. Therefore, recognition of risk factors for progression is of clear benefit. This allows the identification of patients at highest risk, which will benefit most from close monitoring. We hope to improve the rates of early diagnosis with this kind of studies.

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Incidence Of Microbial Colonization And Relevance Of Salivary IgA Estimation In Patients Receiving Chemo-Radio-Therapy For Head And Neck Cancer

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Background: Head and neck cancers make up 4-5% of all cancers. Radiotherapy (RT), on its own or in combination with other treatments like chemotherapy (CT), is an important option in many of these cancers. Microbial colonization/infection of damaged mucosal surfaces by mostly Gram-negative organisms and yeast is a common complication, and this may be exacerbated by concomitant neutropenia. Saliva consists of various chemical components, of which IgA has antimicrobial activity and prevents growth, adherence and aggregation of micro-organisms like various viruses, bacteria and fungi. Aim of this study was to determine the incidence of microbial colonization in Head & Neck cancer patients, post-chemotherapy/radiotherapy and to determine the contribution of levels of salivary immunoglobulin (IgA) on oral health.
Methods:
We examined 150 salivary samples and oral swabs in 50 cases of diagnosed and operated head & neck cancer patients. Swabs & salivary samples were collected before patients were started with either chemotherapy or radiotherapy after their operations to remove the tumour, followed by at 3rd and 6th weeks after chemo/radiotherapy was started. Swabs were processed for microbial culture on blood agar and MacConkey agar. Salivary samples were processed for estimation of IgA on Siemens Dimension analyser using dedicated reagents. Results of microbial culture were documented and compared along with salivary sample reports in pre-chemotherapy and post-chemotherapy groups. Reference range for salivary IgA was considered as 12.43-33.53 mg/dl.
Results:
Of 50 pre-chemo/radiotherapy samples 25 samples showed no growth, 17 samples showed bacterial growth of which Klebsiella pneumoniae was commonest. In 100 post-chemo/radiotherapy samples 33 samples showed no growth and 54 samples showed bacterial growth. When pre and post-chemo/radiotherapy groups were compared for values of IgA, salivary samples showing presence of Klebsiella pneumoniae infection showed significantly increased levels of IgA in post-chemo/radiotherapy group (p=0.0492). In all 150 samples examined, samples with Klebsiella pneumoniae infection (p=0.0022) and all bacterial infection samples (p=0.0426) showed significantly high levels of IgA. When compared to all samples with no growth, IgA levels were significantly high in Klebsiella pneumoniae infection group (p=0.001) and all bacterial samples group (p=0.0005). There was significantly increased incidence of bacterial infection in post-chemo/radiotherapy group (p=0.0233) compared to pre-chemo/radiotherapy group.
Conclusion:
Post-chemo/radiotherapy, there is significantly increased incidence of bacterial infection in head & neck cancer patients. Salivary IgA with its anti-microbial property can be a marker for detection of early infection.

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An ELISA serum assay using monoclonal antibodies against Amyloid beta aggregates

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Alzheimer's disease (AD) is most common dementia. In recent years, the number of AD patients is increasing, because of an aging population. Early diagnosis of AD is desirable for early medical treatment to suppress the progression of the disease. A diagnosis of probable AD is made after medical assessments, and the definitive diagnosis is possible by examining brain tissue after death. Pathological hallmarks of AD brains are the formations of senile plaque (SP) which are mainly extracellular deposition of amyloid β (Aβ). The distribution of SP is classified into three Braak stages (stages A, B and C). Aβ also deposits on the blood vessels of the central nervous

system that is known as cerebral amyloid angiopathy (CAA). Neurofibrillary tangles (NFTs) are intracellular accumulation of hyperphosphorylated tau protein (p-Tau), and its distribution is classified into six Braak NFT stages (I to VI). The reliable biochemical diagnosis is currently low $A\beta_{42}$ levels or elevated levels of p-Tau in cerebrospinal fluid surrounding brain. Here, 392 serums, including those of 376 AD patients and 16 control subjects, of definitively diagnosed individuals through post-mortem examination of the brain were assayed by ELISA. The utilized monoclonal antibodies were 77-3 and 37-11, which react specifically with conformational epitopes on soluble aggregates of $A\beta_{42}$ having diameters greater than 20 and 220 nm, respectively. Using 77-3, higher values were obtained in serum samples from patients with Braak senile plaque stage B (n=24) than from those of the stages 0 (n=25) and C (n=53). Using 37-11, significantly higher than control values were detected in serum samples from patients with moderate-to-very severe cerebral amyloid angiopathy (n=42), a cerebrovascular disorder caused mainly by accumulation of Aβ. No significant differences of the values were detected when patients were classified based on Consortium to Establish a Registry for AD scores or Braak NFT stages. A commercial antibody (82E1) detected little Aβ monomer in sera. These results suggest that ELISA using these antibodies is useful for quick method of diagnosing AD using non-invasive serum.

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Evaluation of the performance of Candida Mannan IgG antibody lateral flow assay for rapid diagnosis of invasive candidiasis

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Background
Despite the recent achievement in disease management, invasive candidiasis is still a life-threatening disease that affects millions of patients worldwide. The objective of this retrospective study was to evaluate the clinical performance of newly developed Candida Mannan IgG lateral flow assay(LFA) in diagnosis of invasive candidiasis.
Material and Methods
Serum samples from 42 adult patients were retrospectively collected in this study. Twenty-three patients had at least one positive candida culture from blood or sterile body fluids, while 19 patients had no clinical signs of candida infection were defined as control group. All patients enrolled in this study were non-neutropenic or immunocompromised. Serum Candida-specific IgG antibody levels were determined by using Candida Mannan IgG antibody LFA (Dynamiker Biotechnology Ltd, China). An additional test was performed if the initial test was positive to confirm the results.
Results
Eighteen of the 23 patients with invasive candidiasis had positive Candida Mannan IgG results, while 5 patients were Candida Mannan IgG positive in control groups. All positive results were confirmed by additional LFA tests. The sensitivity and specificity of Candida Mannan IgG LFA were 78.2% and 73.6%, respectively.
Conclusion
The sensitivity of Candida Mannan IgG LFA was reasonable good, and the specificity was moderate. Considering it only takes 20 minutes to perform the test, the Candida Mannan IgG LFA may provide a rapid diagnostic aid in diagnosis of invasive candidiasis.
Table 1 Evaluation of clinical performance of Candida Manna IgG LFA

	Candida Culture			
	Positive	Negative	Total	
LFA(IgG)	Positive	18	5	23
	Negative	5	14	19
	Total	23	19	42

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Prognostic Value of Pretreatment Albumin to C-reactive Protein Ratio in Patients with Hepatocellular Carcinoma

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Background: Despite the recent advances in hepatocellular carcinoma(HCC) treatment, the prognosis of HCC patients remains substandard. Recent evidence revealed that systemic inflammatory response markers play a key role in the prognosis evaluation. The aim of this study was to investigate the prognostic value of the pretreatment Albumin to C-reactive Protein Ratio (ACR) in HCC patients.
Methods: We retrospectively collected 409 newly diagnosed HCC patients and investigated the correlations among the pretreatment ACR, baseline clinicopathologic features, and overall survival (OS). We applied the X-tile software to determine optimal cut-off points for ACR. A chi-squared test was performed to compare baseline clinicopathologic features in different subgroups, Cox regression and log-rank tests to assess the association of ACR with OS, and Kaplan-Meier curves to estimate OS.
Results: Patients with a lower ACR significantly associated with advanced clinicopathologic parameters and poor OS, with optimal cut-off points of 5.4 (high ACR, n=236 versus low ACR, n=173). Multivariate analysis demonstrated that ACR was associated with OS (hazard ratio (HR): 0.544, 95% confidence interval (CI): 0.385-0.769, p=0.001) along with tumor size (<5cm/ ≧5cm), TNM stage (IandII/IIIandIV), Treatment exposure (Hepatic resection/others), and serum AFP level (<400ng/L/≥400ng/L).
Conclusion: Pretreatment ACR is a convenient and useful parameter for HCC patients predicting OS. Lower ACR was correlated with large tumor size, advanced TNM stage and a high concentration of AFP. If validated, these results may prove to be useful in designing strategies to personalize management approaches among these patients.

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Comparison of Presepsin (PSEP) and Procalcitonin (PCT) for Risk Stratification in the Setting of a Cardiovascular Intensive Care Unit

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Background
PSEP concentrations have been shown to increase as a result of systemic inflammation triggered by bacterial infections. Clinical severity of sepsis and mortality risk can be predicted already by a single determination of PSEP at first presentation to the emergency department.
Objective
The purpose of our study was to investigate whether PCT and PSEP can contribute to detection of sepsis and risk stratification of critical patients from cardiovascular conditions admitted at the intensive care unit (ICU).
Methods
71 patients admitted at the ICU were included in the study. The study examined 4 patient groups: 0: patients with transfemoral implantation of a prostethic aortic valve (TAVI) without evidence of infection or sepsis who served as control group (n=17), 1: patients with sepsis (n=20), 2: patients after sudden cardiac death and resuscitation (n=22), 3: patients with severe pneumonia requiring assisted ventilation (n=12). PSEP and PCT were determined at the time of admission to the ICU by using PATHFAST Presepsin (LSI Medience Corporation, Tokyo) and cobas PCT BRAHMS (Roche Diagnostics). C-reactive Protein (CRP) was measured using the cobas assay (Roche Diagnostics).
Results
The patients with sepsis revealed higher PSEP and PCT values compared to the other patient groups. Discrimination between controls and sepsis revealed RO-AUC values of 0.924 and 0.967, respectively. 23 patients died and 28 patients developed acute kidney injury receiving dialysis. Non-survivors (n=23) and patients with AKI/Dialysis (n=28) showed significantly elevated values. The results are summarized in the table. As CRP is commonly used as inflammatory marker in the ICU we added the CRP values for comparison.
Conclusion
PSEP showed the best diagnostic performance and discriminative power and may be used for risk stratification in general in the ICU setting. PATHFAST PSEP can determined in whole blood within 17 min and is suitable as POC assay in the ICU.

Summary of results						
	AKI, (N=28) Median (IQR)	**Non-AKI**, (N=43) Median (IQR)	**RO-AUC**	**Non-survivors,** (N=23) Median (IQR)	**Survivors,** (N=48) Median (IQR)	**RO-AUC**
PESP, ng/L	2293 (1290-3511)	634 (393-861)	0.855	2462 (1188-3706)	710 (508-1238)	0.798
PCT, ng/ml	8.95 (3.42-26.4)	0.29(0.07-4.27)	0.797	8.77 (3.34-39.4)	0.68 (0.08-6.18)	0.734
CRP, mg/dl	136 (47-204)	44 (13-132)	0.680	134 (50-201)	52 (14-141)	0.650
SOFA score	11.0 (8.0-13.0)	8.0 (1.010.0)	0.718	11.0 (9.0-13.0)	7.0 (1.0-10.0)	0.781

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Implementation of an innovative plasma separation technology enabling improved laboratory efficiency

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Background: Laboratories have many challenges in order to obtain high quality blood samples, generate accurate results and meet turnaround time (TAT) targets. Poor sample quality impacts laboratory operations and quality of results, often requiring manual remediation. A published survey indicated that with a 3% incidence rate, fibrin strands are one of the most common issues. Although some laboratories have implemented significant improvements, including the use of automation and plasma-based samples, challenges still remain. While potentially reducing fibrin-related issues, a change from serum to plasma gel samples has also historically meant reduced analyte stability due to entrapment of cells in plasma above the gel barrier and the potential for instrument interference from the gel remains. The BD Vacutainer® Barricor™ Plasma Blood Collection Tube (BD Barricor) uses an inert mechanical (non-gel) separator technology, creating a high quality plasma sample for a wide range of chemistry applications, with serum-like stability of up to 7 days for many analytes. As a historic serum user with laboratory automation, we partnered with BD to measure the efficiency and economic impact of implementing BD Barricor on a number of laboratory key performance indicators (KPI). **Methods:** A non-randomized, non-interventional, prospective observational study comprised of a 6-month pre-phase, with BD Vacutainer® SST™ II *Advance* Blood Collection Tube (BD SSTII) and a 6-month post-phase, with BD Barricor, conducted in the department of clinical chemistry of the Erasmus Medical Centre (EMC, Rotterdam, The Netherlands). For each phase, KPI which included TAT, defined as receipt in lab to result reported on lab information system (LIS), percentage of achieved STAT TAT goal, sample remediation activities and instrument maintenance and downtime were measured using data from the LIS and time and motion observations. Descriptive statistics and p values were determined to allow comparison of the two phases of the study. Metrics from the EMC lab were used to estimate the opportunity created as a result of the implementation of the new tubes. **Results:** 220,418 pre-phase and 228,796 post-phase tubes were assessed. Implementation of BD Barricor in the post-phase resulted in a TAT reduction of 11.16% across all tubes processed in the laboratory (2.6% for STAT and 13.2% in routine and external samples). This translated to an increase from 78% to 80% tests meeting the current STAT goal of 90 minutes. Sample quality was improved in the post-phase, with the incidence of fibrin, clot or gel-related issues reduced from 3.2% to 0.16%. Data extrapolated over 6 months indicated that there was a 94.8% reduction in remediation activities (7,009 incidences to 365). **Conclusion:** By implementing BD Barricor, we have seen improvements in chemistry sample quality, associated with reduced laboratory TAT and an increase in STAT TAT goal achievement. The enhanced efficiency through shorter TAT and reduction in fibrin, clots and gel-related issues provides an opportunity for the redeployment of valuable resources to other tasks, providing an economic benefit to the lab.

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The Prevalence Of Hypoglycemia In Geriatric Patients With Chronic Kidney Disease

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Background: An estimated 1 in 10 American adults suffer from some degree of Chronic Kidney disease (CKD), along with millions of others at an increased risk of acquiring the disease. The prevalence of CKD increases with age; and it is estimated that more than 40% of adult over the age of 60 years have some degree of CKD. It has been reported that patients with CKD have an increased chance for hypoglycemia than those without CKD. **Methods:** 28,000 specimens were collected from patients residing in long-term care facilities, 11,470 were male and 16,530 were female. Glucose and serum creatinine were measured using Roche/Hitachi P Modular; a eGFR was calculated based on MDRD equation using serum creatinine, age, gender, and race. Patients' data were separated into 5 groups based upon eGFR; and was analyzed further based on gender and age. The prevalence of patients with glucose <50 and <65mg/dL was calculated. Statistical analysis was done using Analyse-it. **Results:** 58% of the patients had eGFR >60 mL/min/1.73 m^2 and the percentage starts declining with age. to reach 23.3% of the patients in the >90 year old group. The prevalence of Glucose <50 mg/dL and <65 mg/dL increased with the decrease in eGFR. Patients older than 81 year old had the highest prevalence of low glucose.

eGFR mL/min/1.73 m^2	Total specimens	Glucose <50 mg/dL	Glucose <65 mg/dL
<15	787	5.3%	16.5%
15-29	2483	2.9%	11.2%
30-44	3813	2.4%	9.4%
45-59	4459	2.0%	8.9%
<60	16460	1.0%	9.1%

Conclusion: Hypoglycemia is more common in patients with a decrease in kidney function, which could be due to very tight glycemic control, a decreased insulin degradation in peripheral tissue, and a prolonged the life of antidiabetic medication due to a decreased renal clearance. Hypoglycemia should be suspected in patients with CKD who present with mental or neurological changes. In addition, diabetic management in these patients should be addressed very cautiously, and one may require adjustments for his or her oral hypoglycemic medication and insulin dosage.

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Combined approach for validation of the pneumatic tube systems

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Background: pneumatic tube system (PTS) is a major transportation route for delivering specimens to the laboratory throughout medical centers. This system offers several advantages such as improving the turn-around times and reduced cost and labor; however, it can cause pre-analytical variations by affecting the quality of blood samples due to acceleration forces, transportation speed, or lack of cushioning inside the sample carriers. In this study, we aim to validate PTS in a recently built hospital in the Emory Healthcare System as well as assess the performance of existing PTSs. **Methods:** we first collected the blood samples from 60 individuals in duplicates and transferred them to the Emory Core Laboratories, one sample via the PTS and one on foot. Samples were tested for 41 analytes. Statistical analysis of differences in obtained test values was performed using a paired Student *t*-test. Moreover, previous studies have shown that the three-axis acceleration/*g*-forces, time and distance have an impact on cell hemolysis. We utilized smartphone accelerometers and data-logger apps to compare the *g*-forces for the phone transferred to the laboratory on foot and via PTS route. **Results:** our results indicate a statistically and clinically significant increase in aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels while transported via the PTS. We applied sponge-rubber inserts inside the carriers to prevent the hemolysis during the transport, which significantly decreased the discrepancy found in LDH and AST values in samples carried via the PTS. The results from our cell phone study also showed that the highest impact on the samples that were hand-delivered (5 *g*) was at least 2 times less than the highest impact seen for samples transferred with PTS (11 *g*). In addition, hand-delivered samples did not have abrupt changes in *g*-forces compared to samples transferred via PTS. **Conclusion:** using a combined approach of testing clinical samples as well as assessing *g*-forces provides hospitals with more detailed assessment of the existing or newly built PTS.

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1/3 hours rule in and rule out algorithm for NSTEMI Using a High-Sensitivity Cardiac Troponin I at Emergency Department in Chinese Population

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Background: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide. Use of high sensitivity cardiac troponin (hs-cTn) assay can improve the early diagnosis of AMI, especially non-ST-elevation myocardial infarction (NSTEMI). Current European Society of Cardiology (ESC) guidelines recommend 0-/1-hour and 0-/3-hour 'Rule-in' and 'rule-out' algorithms for NSTEMI by using hs-cTn. However, it lacks Chinese population data based on such diagnosis process. Thus, this study is to validate 1-hour and 3-hours diagnostic strategy using hs-cTnI (ARCHITECT) in Chinese patients with suspected NSTEMI. **Methods:** From January to December in 2017, 283 patients with suspected ACS presenting to the emergency department were included. Patients aged 18-75 years without STEMI, major operation within 4 weeks, severe renal insufficiency (Ccr <30 ml/min), acute myocarditis or chronic heart failure. Serial measures of hs-TnI level were performed at 0 hour, 1 hour and 3 hours in patients with suspected AMI. The diagnosis of each enrolled patient will be made according to routine clinical approach and 1-hour and 3-hours clinical approach, respectively. The routine clinical diagnosis will be made by cardiologist panel according to third universal definition of myocardial infraction through reviewing all available medical records. The NSTEMI diagnosis depended on hs-cTnI(Architect) assessment will be made a senior cardiologist according to 1-hour and 3-hours clinical approach recommended by 2015 ESC guidelines for the management of NSTEMI. Finally, the positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity are evaluated by using 0-/1-hour and 0-/3 hours algorithm. Statistical analyses were undertaken using MedCalc software version 15.2.2 (MedCalc Software, Mariakerke, Belgium). **Results:** The age of the study population was 59.5 years (95%CI, 58.2-60.8); 91 patients(32.2%) were diagnosed with NSTEMI. The hs-cTnI concentrations of patients with NSTEMI at 0h, 1h and 3h were significantly higher than non-ACS, 3.925ng/mg, 1.065 ng/ml and 0.908 ng/ml respectively. The 0-/1-hour hs-cTnI change was 0.613ng/ml (95% CI, 0.321-0.906), and 0-/3-hour hs-cTnI change was 3.011 ng/ml (95% CI, 0.283 - 5.740). The PPV of 1-hour algorithm was 91.0% and 97.4% for the 3-hours algorithm. The NPV of the 1-hour algorithm was 91.3% and 90.4% for 3-hours algorithm. The sensitivity and specificity was 92.9% and 89.0% for 1-hour algorithm, 87.4% and 98.1% for 3-hour algorithm. When using a baseline hs-cTnI concentration of 0.029ng/ml in male, the PPV is 87.5%, the NPV is 95.5, sensitivity is 93.33%, the specificity is 91.30%. While the PPV in female is 85.7%, the NPV is 95.7%, sensitivity is 92.31%, the specificity is 91.84% using the baseline hs-cTnI concentration of 0.021ng/ml. **Conclusion:** The diagnosis of NSTEMI based on hs-TnI in Chinese patients is similar with pervious studies in European and American population. The application of absolute hs-TnI changes after 1 hour and 3 hours may facilitate rapid rule-in and rule-out of patients at Chinese emergency department.

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Circulating Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) Levels Reflect Renal Function in Newly Diagnosed Patients with Multiple Myeloma Who Are Treated With Bortezomib-Based Therapy

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Background: Renal impairment is a common complication of multiple myeloma. suPAR is the circulating form of a glycosyl-phosphatidylinositol-anchored three domain membrane protein that is expressed on a variety of cells, including immunologically active cells, endothelial cells, and podocytes. suPAR has been implicated in the pathogenesis of kidney disease, specifically focal segmental glomerulosclerosis and diabetic nephropathy, through interference with podocyte migration and apoptosis. We aimed to investigate a possible link between suPAR plasma levels and renal function decline in newly diagnosed patients with symptomatic myeloma before and after frontline therapy with bortezomib-based regimens. Methods: We studied 47 newly-diagnosed MM patients (26M/20F, median age 69.5 years) before the administration of any kind of therapy and after best response to bortezomib-based therapy. Thirty(64%) patients had IgG-myeloma, 7(15%) had IgA and 10(21%) had light-chain only myeloma; 13(28%) patients had ISS-1, 19(40%) ISS-2 and 15(31%) had ISS-3 disease. Twenty-seven(57%) patients had eGFR<60 ml/min/1.73m^2, 23(49%) had eGFR<50 ml/min/1.73m^2 and 10(21%) had eGFR<30 ml/min/1.73m^2; no patient was on dialysis. suPAR concentration was measured in the serum of all patients and of 24 healthy individuals by means of an immunoenzymatic assay (ViroGates, Denmark) along with a series of other blood chemistry markers: of renal function (Cystatin-C) and injury (NGAL); inflammation hs-CRP and IL-6; as well as parameters of cardiac function such as hs-Troponin-T and NT-proBNP. eGFR values were calculated based on CKD-EPI/Cystatin-C equation. Results: We found that suPAR levels were elevated in MM patients at diagnosis compared to healthy individuals (4.1±2.2pg/mL (1.4-13.0pg/mL) vs. 1.8±0.3pg/mL (1.1-2.6pg/mL), p<0.001). Similarly, all other markers of cardio-renal dysfunction and inflammation were elevated in MM patients compared to controls (p<0.01 for all comparisons). suPAR levels strongly correlated with disease stage (ISS-1: 2.4±1.2pg/mL; ISS-2: 3.6±1.8pg/mL and ISS-3: 5.1±2.2pg/mL; p-ANOVA <0.001). After bortezomib-based frontline therapy (VCD=32, VTD=7, VMP=7, VD=1), 9(19%) patients achieved a complete response (CR), 11(23%) very good partial response (vgPR) and 19(40%) PR. Of 23 patients with eGFR<50 ml/min/1.73m^2, 18(78%) showed at least minor renal response to bortezomib-based frontline treatment, according to IMWG criteria. However, at patients' best response no significance changes of suPAR (4.4±2.7pg/mL) levels were observed (p=0.31). On the other hand, suPAR levels both at diagnosis and at best response strongly correlated with eGFR values (r=-0.700, p<0.001 and r=-0.890, p<0.001, respectively) and NGAL levels (r=0.657, p<0.001 and r=0.586, p<0.001, respectively). suPAR levels at diagnosis and at best response also correlated positively with log(IL-6) and log(hs-CRP) values (p<0.001) and markers of cardiac function hs-Troponin-T and NT-proBNP (p<0.001). Conclusions: We conclude that suPAR levels are associated with renal function in patients with multiple myeloma both at diagnosis and at best response to bortezomib-based frontline therapy. Although suPAR correlates with disease stage, confirming previous observations, responders to anti-myeloma therapy continued to have elevated circulating suPAR, possibly reflecting persistent kidney damage, despite their renal response. Furthermore, suPAR correlated with the degree of inflammation and heart dysfunction in these patients. Future studies are needed in order to explore whether changes in suPAR may reflect increased risk for renal failure and/or progression in patients with multiple myeloma.

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Association of oxidative stress and inflammation with the markers of non-invasive peripheral arterial disease in metabolic syndrome

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Background: Oxidative stress and chronic inflammation contribute to the initiation, progression and thrombotic complications of peripheral arterial diseases (PAD). Ankle brachial pressure index (ABPI) and toe brachial pressure index (TBPI) are commonly used non-invasive markers for assessing PAD. The present study aims to find out if novel cardiovascular risk factors: oxidative stress, chronic inflammation and thrombotic markers, are associated with these non-invasive PAD markers in metabolic syndrome. **Methods:** One hundred volunteers with and without diagnosed diabetes and hypertension were recruited. Brachial, ankle and toe blood pressure and anthropometric measurements were performed. Toe brachial pressure was measured from the great toe using SysToe that uses photoplethysmographs. Ankle pressure was measured from the dorsalis pedis by the hand held Doppler probe. Inflammatory markers- hsCRP, thrombotic marker- D-dimer and oxidative stress markers- erythrocyte reduced glutathione (GSH), erythrocyte superoxide dismutase (SOD), and f_2-isoprostanes were analysed from the blood or urine sample of the participants. Data was analysed by IBM SPSS statistics 20. **Results:** 36 out of 100 volunteers were classified under metabolic syndrome using the National Cholesterol Education Panel, Adult Treatment Panel III definition. Jonckheere trend analysis showed that there was a significant linear increase in the level of the TBPI across the quartiles of GSH (p trend < 0.0005) and SOD (p trend =0.009) and a significant linear decrease across the quartile of D-dimer (p trend = 0.007), whereas, ABPI showed a significant linear decrease across the quartiles of GSH (p trend =0.012) only. ABPI and TBPI did not show any significant patterns across the quartiles of hsCRP and f_2-isoprostanes. TBPI was significantly correlated with GSH, SOD, f_2-isoprostanes, D-dimer and hsCRP among volunteers with metabolic syndrome. **Conclusion:** The findings of the present study underscore the potential mechanism of oxidative stress-driven progression of peripheral atherosclerotic disease. Association of ABPI and TBPI with D-dimer in metabolic syndrome group also indicates that increased activation of fibrinolytic system in metabolic syndrome may be associated with the development of PAD.

A-149

Viability assessment of in vitro fertilized embryos using a novel biomarker candidate.

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Objective
Infertility is a spreading phenomenon worldwide resulting in an increasing need for assisted reproduction and *in vitro* fertilization procedures. Since multiple embryo transfer increases the prevalence of multiple gestation, single embryo transfer gains ground. Therefore, finding the embryo with the best implantation potential is crucial. In retrospective experiments a previously identified possible biomarker - the alpha-1 fragment of human haptoglobin (HptA1) - was quantitatively measured in spent culture media of *in vitro* fertilized human embryos.
Relevance
Non-invasive viability assessment from the culture medium of *in vitro* fertilized embryos provides an additional approach to select embryos with the best implantation potential. The field is still developing by mass spectrometric and proteomic approaches. The final goal of the described research is to adapt a complex mass spectrometric assay to a lab-on-a-chip measurement.
Methodology
The study involved 122 patients, aged 26-43 years (mean: 34.4±4.7 years) with a BMI of 17.9-31.6 (mean: 23.2±2.9) representing single and double embryo transfers. HptA1 in spent embryo culture medium samples (n=201) were measured using liquid chromatography coupled mass spectrometry (LC-ESI TOF MS) in retrospective, blind experiments. Haptoglobin and also HptA1 is present in the culture medium and the concentration increases during in vitro embryonic development. Embryos were diagnosed as "non-viable" by the mass spectrometric assay if the amount of HptA1 was elevated with more than 20% compared to the blank control medium, otherwise embryos were assigned as "viable". Samples were divided into two groups: in the control group (n=102) embryos of the patients were assessed using the traditional morphological examination, while in the double-assay group (n=101) embryos were assessed by both the morphological and the mass spectrometric assays. Live birth rates were compared between the two groups.
Validation
In the control group, the embryos were only assessed by the Istanbul Consensus Criteria System ("good" or "fair"). 28 cases of live birth were observed out of 102 transfers meaning a live birth rate of 27.4%. In the double-assay group (n=101) samples of embryos were assessed as "good" or "fair" by the morphological assay as well as assessed "viable" by the mass spectrometric assay. 47 cases of live birth were observed meaning a live birth rate of 46.5%. The difference in the concentration of HptA1 according to outcomes "live-birth" and "no-birth" was significant ($p<0.001$). The clinical sensitivity was 100%, while specificity 55%, area under ROC curve was 0.906.
Conclusions
The increased amount of HptA1 in culture media samples of *in vitro* fertilized embryos negatively correlates with implantation potential. By combining the traditional morphological evaluation with the mass spectrometric assay, an increment in live birth rate was found in retrospective experiments. The HptA1 assay might serve as an additional tool to increase success rate of *in vitro* fertilization.

A-150

Evaluation of the Modified Carbapenem Inactivation Method for Carbapenemase Activity Assay in Carbapenemase-producing *Enterobacteriaceae*

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Background: Carbapenems are commonly used as primary option for treatment of multi-drug resistant *Enterobacteriaceae*, that and are often considered last-line antibiotics. The emergence and dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE) are significant clinical in public health concern. Therefore, correct detection of CPE is essential for determining appropriate antimicrobial therapy and infection control measures to avoid spread and potential outbreaks. Although several tests are described for the screening and detection of carbapenemases, there are limitations in each method for identification of CPE, such as requiring special reagent or equipment. Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S27) reported modified carbapenem inactivation method (mCIM) has been developed in 2017 as a phenotypic technique for detecting carbapenemase activity. In this study, we evaluated the carbapenemase activity with mCIM for routine assay in laboratory to detect CPEs from clinical specimens. **Methods:** The clinical specimens were collected from August 2011 to Dec 2017 and tested for drug susceptibility to imipenem, meropenem, and ertapenem using disk diffusion method. The production of carbapenemase was detected by mCIM that contents of a 10 μg meropenem (MEM) disk was degraded through carbapenemase activity when the disk was incubated in a bacterial suspension of CPE. MEM degradation was assessed by subsequently incubating the disk on a lawn of a sensitive *Escherichia coli* indicator strain. Using specific primers were confirmed carbapenemase genes (KPC, NDM, IMP, VIM, and OXA-48) by multiplex polymerase chain reaction (PCR). The mCIM results were compared with multiplex PCR. **Results:** A total of 320 CPE clinical specimens was isolated and analyzed from En Chu Kong hospital in Taiwan. Among those bacterial strains, the most common species were *Klebsiella pneumoniae* (n=194), followed by *Escherichia coli* (n=40), *Morganella morganii* (n=30), and other species (n=56). We found out there were fifteen strains of KPC gene, nine strains of IMP gene, two strains of VIM gene, two strains of NDM-1 gene, ten strains of OXA-48 gene detected in samples, and one strain simultaneously expressed KPC and OXA-48 genes. All multiplex PCR positive strains showed carbapenemase activity by mCIM, except two false-negative results for OXA-48 producers (bacteria strains) that were further confirmed by sequencing and BLAST alignment of National Center for Biotechnology Information of the United States National Library of Medicine. CLSI guidelines reported mCIM > 99% sensitivity and specificity for CPE detection. In this study, the sensitivity and specificity of mCIM were up to 95% and 100%, respectively. **Conclusion:** Our research showed higher performance (sensitivity/specificity) in mCIM for CPE detection which the test procedures are simple without special reagents, equipments, or technique. Thus mCIM is suitable screen test of carbapenemase activity assay in microbiological laboratories.

A-151

Clinical evaluation of a rapid fully-automated multiplex biochip array for Stroke diagnosis

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Background: Stroke is a cerebrovascular event, which impedes or reduces blood supply to the brain resulting in localized cell death. Haemorrhagic stroke (HS) describes the rupture of a cerebral artery resulting in intracranial bleeding, whilst an ischemic stroke (IS) describes thrombolytic occlusion in a cerebral artery resulting in ischemia. Transient ischemic attack (TIA) - defines a transitory disruption of the blood flow to the brain, which prevails for less than 24 hours. The accurate and timely diagnosis of stroke subtype is critical for determining an effective treatment strategy, which ultimately impacts patient prognosis and survival. However, accurate diagnosis and classification of stroke subtype currently presents a significant clinical challenge, and represents an unmet clinical need. Recent advances in clinical research have identified biomarkers with the potential to assist clinicians in diagnosing and classifying stroke. Randox presents a multiplex biochip array hosting a panel of 6 biomarkers - D-dimer, Soluble Tumour Necrosis Factor Receptor 1 (sTNF-R1), Parkinson disease protein 7 (PARK 7), Glial Fibrillary Acidic Protein (GFAP), Interleukin 6 (IL-6) and Fatty Acid Binding Protein 3 (FABP3) - aimed at the rapid diagnosis and differentiation of stroke. The aim of this study is to demonstrate the utility of a multiplex biochip array, incorporating a collection of novel biomarkers, to rapidly diagnose and differentiate stroke subtypes. **Methods:** A cohort of 192 samples (EDTA plasma) including 76 acute stroke patients following hospital admission (53 confirmed IS; 10 HS; 13 TIA), 37 stroke mimics and 79 controls were tested using the Randox Stroke Array. The methodology utilizes simultaneous chemiluminescent sandwich immunoassays immobilized at discrete test regions on the biochip surface. The array was applied to the new, fully automated Evidence Evolution analyser, which can produce the first set of results within 36 minutes, and one set of results per minute thereafter, enabling efficient, automated sample analysis. **Results:** Elevated biomarker levels were observed in stroke samples compared to normal controls - D-dimer (AUC = 0.957; $p < 0.001$), FABP3 (AUC = 0.926; $p < 0.001$), IL-6 (AUC = 0.917; $p < 0.001$), sTNF-R1 (AUC = 0.855; $p < 0.001$) and PARK 7 (AUC = 0.826; $p < 0.001$). Furthermore, the Randox Stroke Array successfully differentiated stroke patients from stroke mimics (e.g. hypoglycaemia, hyponatraemia, seizures, migraines, brain tumour, subdural haematoma or brain tumour) - D-dimer (AUC = 0.839; $p < 0.001$), FABP3 (AUC = 0.821; $p < 0.001$), IL-6 (AUC = 0.75; $p < 0.001$), PARK 7 (AUC = 0.852; $p < 0.001$) and sTNF-R1 (AUC = 0.78; $p < 0.001$). Significantly, plasma GFAP levels were increased in HS patients compared to IS patients (AUC = 0.902; $p < 0.001$) indicating the potential

of this marker to distinguish between IS and HS. **Conclusion:** These findings demonstrate that the Randox Stroke Array can be utilized to reliably diagnose and differentiate stroke subtypes, in an efficient manner using the fully automated, Evidence Evolution analyser. This advancement is poised to become an invaluable adjunctive diagnostic tool in the diagnosis and treatment of stroke.

Tuesday, July 31, 2018

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

Electrolytes/Blood Gas/Metabolites

A-152

Study Of The Effect Of Storage Temperature And Serum-Clot Contact Time On Serum Sodium And Potassium Levels

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Background:
Sodium (Na^+) and potassium (K^+) are the most commonly measured electrolytes in the clinical laboratory. The energy dependent, sodium-potassium pump is the principle mechanism for active transport of these ions across cell membranes in-vivo. The existing glucose is spent over time in-vitro and leads to Na^+/K^+ pump failure when the separation of the blood clot from serum is delayed. Further delay in separation leads to passive diffusion of K^+ out of cells and sodium into the cells causing changes in the serum K^+ and Na^+ concentrations. Different shifts of Na^+ and K^+ have been observed when whole blood is stored at different temperatures. The delay in transport of samples from the site of collection and processing in the laboratory due to various reasons has been observed. Transport and storage of these samples at different temperatures are also not uncommon.
Objectives:
1.To find out the maximum, acceptable time delay between collection of blood and separation of serum and the optimum storage temperature that should be maintained during this period of delay for serum sodium and potassium assays. 2. To study the time and temperature dependent changes of serum potassium and sodium concentrations during this period of delay.
Method:
A descriptive cross-sectional study was performed using 50 volunteers who had been requested for serum sodium and potassium assays. Each specimen was analyzed using direct ISE method at different serum-clot contact time i.e.1, 2,3,4,6 & 24 hours and at 21-25^0 C and 2-8^0 C storage temperatures. All Quality management procedures were implemented during the analysis.
Results:
Serum potassium was initially decreased and then increased after 6 hours of serum-clot contact time and at 21-25°C and 2-8°C storage temperatures. But the initial decrease was not statistically significant ($p > 0.05$). Potassium was significantly increased at 24 hour of serum-clot contact time at both storage temperatures ($p<0.05$). There were a 16% increase of the serum potassium level at 21-25°C and a 36 % increase of the potassium level at 2-8°C after 24 hour of serum-clot contact time. The changes of serum sodium level at different serum-clot contact times and storage temperatures were statistically not significant ($p > 0.05$).
Conclusion:
The samples for serum electrolytes should be separated from the blood clot before 6 hours since collection and preferably stored at room temperature (21-25^{0}C) until such time. However, further studies are required to investigate the effect of serum-clot contact time at different points of 6 to 24 hour time interval which was not tested during this study to come to a conclusion on maximum acceptable period of delay in serum separation.

A-153

Rapid determination of serotonin in human serum by ultra-performance liquid chromatography with fluorescence detection.

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Background: Serotonin is an important biogenic amine involved in the regulation of several physiological functions. The main diseases associated to high serum serotonin level are neuroectodermal tumors as carcinoid tumor. The objective of this work was to develop a simple and fast method for determination of serotonin in serum by UPLC with fluorescence detection for clinical diagnosis. **Methods:** 500 µL of serum were precipitated with 500 µL of trichloroacetic acid 10%. The solution was mixed for 60 seconds and centrifuged at 3000 rpm for 10 minutes. 600 µL of the supernatant were transferred to a glass tube and 400 µL of Tris(hydroxymethyl) aminomethane 1.0 mol.L^{-1} solution were added. Chromatography was performed on an Acquity UPLC system (Waters) equipped with an Acquity BEH C18 column (50 mm x 2.1 mm x 1.7 µm) - Waters held at 30°C and isocratic mobile phase. Detection was performed on a Waters fluorescence detector operated with excitation at 292 nm and emission at 337 nm. **Results**: The chromatographic run time was approximately 1.5 min. Linear range obtained from 20 to 1000.0 ng.mL^{-1} and dilution was validated for samples that exceed the curve in 4 times. The calculated Limit of detection was 6.8 ng.mL^{-1}.Imprecision intra-day was less than 1.5 % and inter-day was less than 3.8%. **Conclusion**: The UPLC method has been developed and validated successfully for the quantitative analysis of serotonin in serum and has been implemented in clinical routine laboratory.

A-154

Performance Evaluation of the Atellica CH Ca, GluH_3, K, Na, Cl, CO2, UN_c, and Crea_2 Assays versus the Dimension EXL Assays

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Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH Analyzer vs. the Dimension® EXL™ Integrated Chemistry System for various chemistry assays, including Calcium (Ca), Glucose Hexokinase (GluH_3), Potassium (K), Sodium (Na), Chloride (Cl), Carbon Dioxide (CO_2), Urea Nitrogen (UN_c), and Creatinine (Crea_2). These assays are among the most commonly ordered tests in hospitals and outpatient clinics, as they provide a broad snapshot of the patient's current health. **Method:** Method comparison was used to evaluate performance. Studies were conducted according to CLSI EP09-A3, with patient-sample results compared to results from the Dimension EXL system. **Results:**

Assay	Regression Equation	r	Comparative Assay
Ca (serum)	y = 1.05x - 0.7 mg/dL	0.998	Dimension EXL CA
Ca (urine)	y = 1.07x - 1.3 mg/dL	0.996	Dimension EXL CA
GluH_3 (serum)	y = 0.97x - 5 mg/dL	0.998	Dimension EXL GLUC
GluH_3 (urine)	y = 1.00x - 7 mg/dL	0.991	Dimension EXL GLUC
GluH_3 (CSF)	y = 0.97x + 1 mg/dL	0.991	Dimension EXL GLUC
K (serum)	y = 0.93x + 0.2 mmol/L	0.999	Dimension EXL K
K (urine)	y = 1.09x - 0.8 mmol/L	1.000	Dimension EXL K
Na (serum)	y = 1.00x - 1 mmol/L	0.998	Dimension EXL NA
Na (urine)	y = 1.11x - 2 mmol/L	0.999	Dimension EXL NA
Cl (serum)	y = 1.00x + 2 mmol/L	0.996	Dimension EXL Cl
Cl (urine)	y = 0.98x + 0 mmol/L	0.997	Dimension EXL Cl
CO_2	y = 1.10x + 0 mEq/L	0.989	Dimension EXL ECO_2
UN_c (serum)	y = 1.03x + 0 mg/dL	0.999	Dimension EXL BUN
UN_c (urine)	y = 0.93x - 10 mg/dL	0.999	Dimension EXL BUN
Crea_2 (serum)	y = 0.98x - 0.02 mg/dL	1.000	Dimension EXL CREA
Crea_2 (urine)	y = 0.87x + 3.45 mg/dL	0.998	Dimension EXL CREA

Conclusions: Method comparison results for these chemistry assays showed acceptable agreement with an on-market comparative analyzer.

A-155

Ion measurement by direct ISE vs. indirect ISE. Analytical performance evaluation according to different quality requirements.

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Background: For patient safety, medical laboratories must offer accuracy in their results. Small results variation for sodium, potassium and chloride can lead to incorrect patient evaluation or treatment. The most widely used method is ion selective electrode (ISE), by direct potentiometry (direct ISE) or indirect potentiometry (indirect ISE). **Objective:** To evaluate and compare the performance of direct and indirect ISE for three ions: sodium (Na), potassium (K) and chloride (Cl), in terms of Total

Error (TE) and sigma performance (σ). **Materials and methods:** Retrospective study based on internal quality control data recorded in Unity Real Time® interlaboratory program (BioRad) during August 2017 to January 2018. Each analyte's laboratory and peer group mean (x) and standard deviation (s) were obtained for two concentration levels of control samples: normal (N) and pathological (P). They were measured by indirect ISE in three Cobas c501 autoanalyzers and in two blood gas platforms by direct ISE, Cobas b221 from Roche Diagnostics (Mannheim, Germany) at two CEMIC´s University Hospitals. Total laboratory error (TE_L) and 6 Sigma performance (σ) were calculated for each analyzer. For methods' performance evaluation for each analyte, TE_L was compared to allowable CLIA total error (TE_a) and Biological Variation (BV) requirements. Method decision graphs combining BV specifications and 6 Sigma model were prepared with calculated imprecision and bias data. **Results:** For the two methods in both concentration levels, K reached laboratory established TEa (0.5 mEq/L) and presented an acceptable sigma value, greater than 6 for the two direct ISE analyzers; for indirect ISE, the obtained sigma was between 3 and 5. BV minimum requirement (8.4 %) was reached by all methods. For Na, only the two direct ISE analyzers reached the established TEa (4mEq/L) and presented an acceptable Sigma, between 4 and 6 Sigma, both for normal and pathological levels. BV minimum requirement (1.1 %) was not reached by any method. Cl had a similar behavior to Na. TEa (5.0%) was reached for P level only by direct ISE methods showing an acceptable Sigma, between 4 and 5. BV minimum requirement (2.2 %) was not reached. Method decision graphs showed that for Na and Cl, BV minimal requirements can only be reached by analytical procedures that present 6 Sigma performance. **Conclusion:** Routine laboratories' methodologies available today for ion measurement do not always meet the established quality specifications. Laboratories must monitor methods' performance to evaluate error and sigma performance over time. As results reflect the state of the art for these ions' measurement systems, manufacturers are responsible for the improvement of the methods they offer.

A-156

Correlation of Serum Ionized Calcium to Corrected Total Calcium Generated by Two Different Formulae

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Background
In many centres within Nigeria, evaluation of calcium levels is undertaken in the form of serum/plasma total calcium(tCa). Although there is increasing availability of ion selective electrodes for ionized calcium(iCa) estimation, few centres routinely measure it. In view of the role of plasma albumin levels on tCa, formulae are used to generate corrected serum/plasma total calcium(ctCa)**,** for proper clinical interpretation. Traditionally, the conventional formula, attributed to Payne, has been used across many centres but about a decade ago, locally derived formulae that utilized albumin and total protein respectively, was published with little adaptability to clinical practice. There is a need to assess the degree of agreement between serum the physiologically active ionized calcium and the corrected total plasma calcium using the conventional and locally derived formulae respectively, as this can impact clinical interpretations of calcium status especially in the pregnant state. In this study we set out to determine the degree of correlation between measured serum iCa and ctCa derived from three different formulae adjusting for serum albumin concentration or total protein..
Methods
Two hundred and forty apparently healthy women of reproductive age attending six primary-level health care facilities in Abuja, Nigeria were recruited for the study. Ethical clearance was gotten from Federal Capital Development Authority ethical review committee. Blood samples were drawn after obtaining informed consent, in glass syringes and serum separator bottles. Laboratory analyses were by ion-selective electrode method(pH and ionized calcium) and O-cresolphthalein complex method(total calcium). The conventional formula by Payne utilizing serum albumin concentration and two locally derived formulae by Ogunkolo involving serum albumin and total protein respectively were used to generate ctCa. Analysis was by Pearson's correlation.
Results: The mean percentage of ionized calcium (iCa) to corrected total Ca (corrTCa) was 43.7% , though the range of percentage of iCa) to ctCa was across a wide spectrum of subject values (26-65%.) Assessment of the association of iCa to ctCa values showed positive correlation (r= 0.54, 0.41 & 0.29) for the three different formulae but none showed a strong correlation. However the "best fit" or highest correlation coefficient was noted with the conventional formula of Payne, though it was only a fair level of association(r= 0.54). The locally derived formulae by Ogunkolo had lower correlation coefficients than the former; in fact the formula utilizing total protein instead of albumin, was very weak in terms of correlation to iCa.(r=0.29 vs 0.41).
Conclusion: These study findings are in agreement with many other studies which have shown poor correlation between iCa and ctCa, underscoring the importance of measurement of iCa especially in severe and critical disorders where efficient calcium status determination is vital. However, in the event that measurement of iCa is not feasible, it is important that tCa measurement be corrected/adjusted by validated formulae suited to the given environment. This is imperative because it has been shown that ctCa -derived equations using local laboratory data may differ from previously published equations as was the case in our study.

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Comparison of Five Automated Serum Ferritin Immunoassays

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Background: Serum ferritin tests measure the amount of stored iron in the body and can be used for diagnosing iron-related disorders. The objective of this study was to compare the analytical performance of five automated immunoassays for the quantitation of serum ferritin. Linearity, precision, and method comparison studies were performed with the Siemens ADVIA Centaur, Beckman AU5800, Abbott Architect i2000, Siemens Immulite 2000 and Siemens BNII methods. Additionally, recovery of the World Health Organization's 3rd international standard (WHO IS) for ferritin (94/572) was assessed to further compare the performance of methods standardized to different generations of WHO reference materials. **Methods:** Linearity was evaluated by combining two serum patient pools, one with a high ferritin concentration and the other with a low concentration, to create several samples with final concentrations that spanned the analytical measurement range (AMR). Precision studies were performed using 3 levels of MAS Liquimmune QC material (Thermo Fisher Scientific, Waltham, MA, USA). Patient specimens with similar ferritin concentrations were pooled to generate 40 samples with enough volume to be run on all 5 test methods in duplicate. Recovery studies were performed by running the WHO 3rd ferritin IS straight as well as spiking it into serum at varying concentrations to determine if the percent recovery was consistent across the measurable range of the assay. **Results:** Target values for the linearity samples were individually calculated for each method using the lowest and highest measured concentrations that fell within the AMR. Linear regression analysis revealed that all 5 methods had similar slopes ranging from 1.00-1.04, intercepts ranging from -2.60 to 30.52, and correlation coefficients of 0.99. The within run and total imprecision was acceptable (coefficient of variation <10%) for all methods. Patient sample correlations revealed calibration differences that were most apparent between methods standardized to the 1st and 2nd WHO IS. Recoveries of the 3rd IS were 166%-187% for the method claiming traceability to the 1st IS (Architect), 94%-125% for methods claiming traceability to the 2nd IS (Centaur, Immulite, BNII), and 98%-109% for the method claiming traceability to the 3rd IS (AU). The Centaur and Immulite recovery data demonstrated greater recovery at higher ferritin concentrations. For the Architect and AU methods, decreased recovery was observed as the ferritin concentration increased. No appreciable ferritin recovery trend was noted for the BNII. **Conclusion:** Overall, the 5 immunoassays correlated well with each other despite being standardized to different generations of the WHO ferritin reference materials. The small differences observed in ferritin concentrations can likely be attributed to differences in calibrator standardization, antibody specificity, and ferritin isoform composition. The performance of the Architect method was the most different from the group, demonstrating a positive bias for patient samples relative to the group mean as the sample concentration increased as well as unusually high recoveries of the IS.

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Study of The Outcome Of Dysnatremia In ICU Hospitalized Patients

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Background: Dysnatremia is one of the common electrolyte abnormality in clinical settings either in general admissions or in ICU, with more prevalent in ICU settings. Dysnatremia if not promptly address may result in the increase of patient's morbidity, mortality as well as the total duration of hospital stay. Hypo/hypernatremia can occur due to variety of clinical conditions and also due to iatrogenic causes during patient's hospital stay. This study aims at revealing the frequency of dysnatremia in our ICU settings, the common etiologies behind this abnormality along with the length of ICU stay as well as mortality associated with this disorder. **Method:** A total of 102 patients admitted under ICU, in Tribhuvan Univer-

sity Teaching Hospital, were enrolled in this study over 6 months period. Patients fulfilling the inclusion criteria were involved. SPSS ver. 21.0 was used to analyze the data. ANOVA was used to find mean differences and Spearman's, chi square tests was used to establish the correlation between study variables. **Results:** Of total 102 patients with minimum age 18 years and maximum age of 88 years (mean age 57.78 ± 16.64 years), 65 (64%) patients were male and 37 (36%) were females. 40.1% of study population was found to have hypertension and 26.5% were found to have diabetes mellitus. The frequency of dysnatremia in this study was 0.225 (22.5%). Hypernatremia was present in 21% of cases and hypernatremia was seen in 2%. The mean serum potassium level was found to be higher in patients with dysnatremia than in eunatremic patients. No statistical significance was seen between dysnatremia and the comorbid conditions in this study. The mean duration of ICU stay for patients with normal serum sodium level was 5.01±0.83 and for patients with dysnatremia was 6.69±1.9. To evaluate the correlation between dysnatremia and length of ICU stay, spearman's correlation was used, which was statistically significant. Most of the patients with dysnatremia were asymptomatic (52.2%). However in symptomatic patients (47.8%), the most common symptom was confusion (54.5%). In this study, Central Nervous system was involved most which was present in 46% of study population and respiratory system involvement was found in 20%, which represented the second most common system involved. Dysnatremia was most commonly associated with stroke (33%). Strong association was also seen with Pneumonia with severe sepsis (20%). Malignancy (GI/lung/Brain) were seen in 12% of the dysnatremic population. Mortality rates associated with dysnatremia comprised 21.7% of the dysnatremic study group as compared to 17.6% of total ICU mortality. **Conclusion:** This study showed that dysnatremia occurs in ICU hospitalized patients and the length of ICU stays increases with this electrolyte abnormality. So prompt identification and management of dysnatremia should be done. However, further studies are required to reinforce this idea and the effects of early treatment of dysnatremia in ICU patients should be clarified in a prospective interventional trial.

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Discrepancies in Electrolyte Measurements by Direct and Indirect Ion Selective Electrodes due to Interferences by Proteins and Lipids

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Background: Modern laboratories use both direct and indirect Ion selective electrodes (dISE and iISE) for electrolytes estimation and often use the results interchangeably. However, studies report discrepancy in results between the two methods, mostly due to higher protein or lipid levels. However, no study reports the combined effects of proteins and lipids on electrolyte measurement. Here we study the effect of high protein and lipid levels simultaneously, on sodium (Na) and Potassium (K) levels obtained by dISE and iISE in patient samples. **Methods:** 195 serum samples were analyzed for Na and K on Roche Modular P800 by iISE and on XI-921, Caretium by dISE. Serum total protein, cholesterol and triglyceride were measured colorimetrically on Roche Modular P800. Percentage difference was calculated for serum sodium [%Diff_Na = (Na^+_{dISE}- Na^+_{iISE})*100/ Na^+_{dISE}] and similarly for potassium. Comparison was done between patient subgroups with high or normal serum proteins and lipids using Mann Whitney U test. **Results:** Table1 shows the percent differences obtained between dISE and iISE in Na and K estimations. Subgrouping was done on the basis of cut-offs of serum protein (8g/dL), cholesterol (300mg/dL) and triglycerides (<300mg/dL) levels. Significant %_Diff were observed for both Na (p= 0.005) and K (p=0.003) levels by dISE and iISE between samples with protein levels <8g/dL and ≥8g/dL. However, effect of triglyceride levels were evident only on %Diff_K (p=0.047). Cholesterol levels did not affect the %Diffs significantly nor did the combined effect of both lipids. However, %Diffs of both Na and K were found to be significantly affected by levels of protein and lipids when considered together. **Conclusion**

Summarily, interchangeable use of electrolyte results from direct and indirect ISE is not advisable in a setting of hyperproteinemia (≥8g/dL) or hypertriglyceridemia (≥300mg/dL), more so when they are coexistent.

Table 1: %Differences obtained between direct and indirect ISE electrolyte estimations in patient subgroups

Patient subgroups	No. of patients	%Diff_Na	p-value	%Diff_K	p-value
S. TG <300 mg/dL	139	3.57 (-9.94, 13.92)	0.292	2.22 (-13.51, 16.67)	0.047
S. TG >=300mg/dL	56	4.38 (-4.74, 10.13)		3.96 (-5.71, 14.81)	
S. Chol <300 mg/dL	150	3.68 (-9.94, 13.92)	0.312	2.46 (-13.51, 16.67)	0.787
S. Chol >=300mg/dL	45	4.10 (-4.74, 10.33)		3.33 (-5.71, 14.81)	
S. protein <8g/dL	139	3.43 (-4.74, 10.33)	0.005	2.22 (-13.51, 16.67)	0.003
S. protein >=8g/dL	56	5.05 (-9.94, 13.92)		4.59 (-8.00, 16.07)	
S. Lipids <300mg/dL	125	3.57 (-9.94,13.92)	0.282	2.43 (-13.51, 16.67)	0.197
S. Lipids >=300mg/dL	70	4.14 (-4.74, 10.33)		3.39 (-5.71, 14.81)	
S. Protein OR S. Lipids high	124	4.41 (-9.94, 13.92)	<0.001	4.12 (-8, 16.07)	<0.001
S. Protein & S. Lipids normal	71	2.65 (-4.32, 9.49)		0.00 (-13.51, 16.67)	

A-160

Long-Term Sigma Metrics for Na, K and Cl Assays on Abbott Clinical Chemistry Analyzers Based on External Proficiency Surveys

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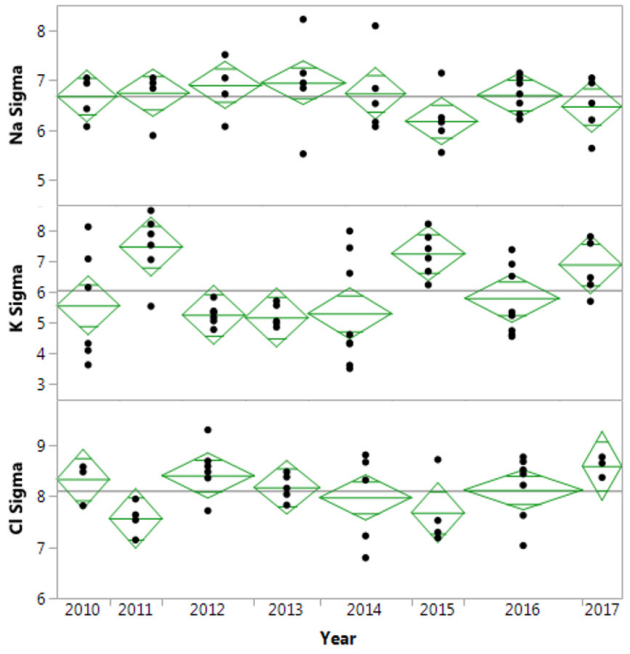
Background: Abbott clinical chemistry analyzers, including ARCHITECT® and Alinity™, use Integrated Chip Technology (ICT) consisting of solid-state ion-selective electrodes (ISE) to measure sodium, potassium and chloride simultaneously in serum, plasma or urine samples. The objective of this study was to assess the long term analytical performance of Abbott serum ICT assays (Na^+, K^+ and Cl^-) using Sigma Metric Analysis based on results from External Quality Assurance (EQA) surveys. **Methods:** Proficiency testing data for Abbott ARCHITECT were obtained from an American EQA program from 2010 to 2017. Sigma metrics were calculated using the equation: Sigma= (TEa(%)-Bias(%))/CV(%), per Westgard QC using RiliBäk TEa targets, where the bias was estimated by comparing Abbott ARCHITECT group mean with ISE diluted Method Mean, and the CV was from Abbott ARCHITECT group with an average of 425 participants. As the Method Mean is mainly determined by the other 90% of non-Abbott ARCHITECT participants, the bias may be overestimated. **Results:** The figure shows the sigma value for each proficiency sample. In serum sodium normal range (136-145 mmol/L), Abbott ARCHITECT Sodium assay had sigma values ranging from 5.5 to 8.2, with an average sigma of 6.7. In serum potassium normal range (3.5-5.1 mmol/L), Abbott ARCHITECT Potassium assay demonstrated sigma values ranging from 3.5 to 8.7 with an average sigma of 6.1. In serum chloride normal range (98-107 mmol/L), the sigma metrics for Abbott ARCHITECT Chloride assay ranged from 6.8-9.3 with an average value of 8.1. **Conclusion:** For normal serum proficiency samples over the studied period of 8 years, Abbott ICT assays demonstrated greater than 6 sigma performance on average, which translates to World Class Quality. This indicates that ICT assays on Abbott Clinical Chemistry Systems consistently provide sodium, potassium and chloride results with excellent accuracy and precision, and contribute to the delivery of measurably better healthcare.



A-161

Evaluation of a serum ammonia assay for urinary ammonium measurement to assess renal acidification impairment

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Background: Urinary ammonium (NH_4^+) output can help predict clinical outcomes in hypertensive kidney disease (Raphael et al., JASN, 2017). Low NH_4^+ excretion is associated with impaired renal acidification and subsequent development of acidosis. Direct measurements of urinary NH_4^+ are more accurate than NH_4^+ estimates from urine anion and osmolar gaps. While FDA-approved urinary NH_4^+ assays are not readily available on automated chemistry analyzers, existing serum ammonium assays can be adapted by implementing an on-board sample dilution. For clinical relevancy, a urine NH_4^+ assay should have a measurement interval of 1-50 mmol/L. In this study, a preliminary validation of a routinely available serum ammonium assay (Randox, Ireland) was conducted on an Architect *ci*8200 analyzer for the purpose of urinary NH_4^+ measurement. **Methods:** For measurement interval determination, a specimen dilution of 1:40 was utilized. Precision (4 days, n = 15 at each level (EL)), linearity (n=3 EL), recovery (n=3 EL), reportable range (n=3 EL) and limit of quantitation (n = 15 EL) were assessed by analyzing 7 NH_4^+ levels (0.7 – 45.0 mmol/L) in 0.9% saline. Recovery studies (n = 3 EL) were conducted by spiking 6 NH_4^+ levels (0.7 – 22.5 mmol/L) into patient urine matrices. **Results:** Precision (%CV) was determined to be < 20% for values 1.4 – 3.4 mmol/L and < 6% for values 3.5 – 44.2 mmol/L. The limit of quantitation (20% CV threshold) was 1.4 mmol/L. The assay was determined to be linear in the range of 0.7 – 45.0 mmol/L with a slope of 0.99 and intercept of 0.79. Recovery in saline was 126% - 131% at 1.4 – 2.8 mmol/L and 118% - 98% at 5.6 – 45.0 mmol/L; however, recovery in urine was 97% - 114% at 1.4 – 2.8 mmol/L and 114% - 117% at 5.6 – 22.5 mmol/L. The acceptable measurement interval (total allowable error of 1.5 mmol/L or 10%; LOQ set at 20% CV) was determined to be 1.4 – 45.0 mmol/L. **Conclusion:** Preliminary investigation demonstrated adequate performance of the Randox assay for determination of urinary ammonium levels. To fully validate this assay for experimental use, a matrix-appropriate evaluation and accuracy assessment against a clinically validated method will be required.

A-162

Use of a new data mining technique demonstrates highly predictable periods of accurate and less accurate point of care testing

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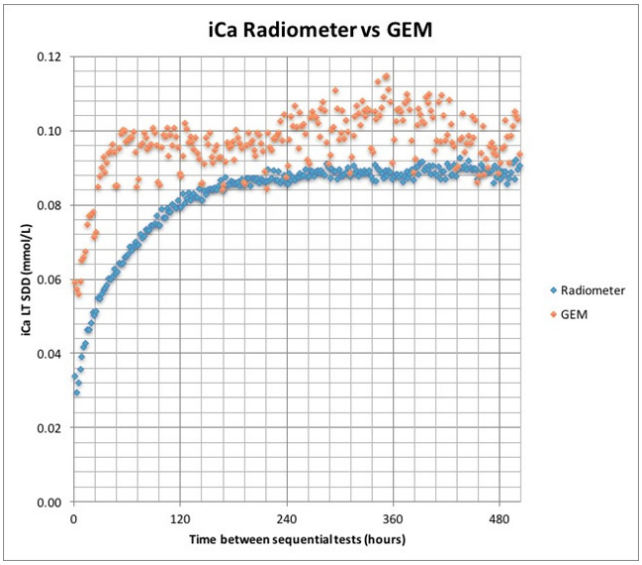
Introduction: Analytical systems with built in quality control (QC) tend to analyze minimal external QC due to the additional expense and effort as well as the disconnect between instrument status and out of limits external QC. For many of these devices, the user has little comprehension of the internal QC error detection capabilities. **Methods and Materials:** We have developed a methodology that evaluates the variation of repeated, sequential intra-patient data to yield measures of biologic, preanalytic and analytic variation. The method involves procuring large series of patient data available in laboratory information systems and grouping consecutive intra-patient result pairs into period bins that reflect the interval of time between consecutive tests. **Results:** The Figure shows the long term variation as measured by the standard deviation of duplicates (SDD) for all possible within patient iCa pairs separated by 2 hour time intervals from 2 hours to 500 hours. The two years of patient data were those of intensive care unit (ICU) patients from the Calgary Foothills Hospital or the Edmonton University of Alberta Hospital who had blood gases and electrolytes measured by tandem Instrumentation Laboratory GEM 4000 or Radiometer ABL 800 instruments, respectively. The Figure demonstrates distinct patterns: 1) the lower variation of the Radiometer iCa, 2) the higher variation at the shortest interval for both the Radiometer and GEM, 3) the regular increase in variation of the Radiometer, 4) regular, short term 24 hour decrements in the GEM that approach those of the Radiometer and 5) generally increased variation in the GEM beginning at the tenth day.**Conclusions:** For the periodic (every 24 hours) ability of the GEM systems to achieve the low Radiometers' variation, we hypothesize that the intermittent, excellent variation is associated with a process that is repeated every 24 hours and coincides with the ICU's early morning run of patient samples.



A-163

An equation for correction of potassium in hemolyzed specimens

D. Wang, N. Babic. *Medical University of South Carolina, Charleston, SC*

Background: Clinical laboratory tests play a significant role in medical decision making. Of the factors that may affect accuracy of laboratory results, in vitro hemolysis is the most frequently encountered. While several analytes are subject to such interference, potassium (K) is probably the most widely recognized one. In this study we developed and validated a simple equation that may be used to accurately estimate actual K concentration in hemolyzed specimens. **Methods**: Proposed equation is: eK= K-HI/([Hgb]*10)*Hct, where K is measured potassium, HI is hemolysis index, corresponding to plasma hemoglobin concentration in mg/dL, Hgb is whole blood hemoglobin in g/dL and Hct is hematocrit. A total of 1072 de-identified, residual pa-

tient samples collected in lithium heparin plasma separator tubes were used for this study. The specimens were split into two major groups: a baseline group (n = 544), with minimal to no hemolysis interference (HI <100), and a test group (n = 528), consisting of matched hemolyzed samples with HI 100-500. The eK values for test samples within 0.5 mmol/L of corresponding baseline K values were considered acceptable, as per CLIA defined total allowable error (TAE). To ensure that eK values are not skewed by external factors that may change K level in vivo, we generated a subset of 72 matched patient samples, excluding patients on KCl treatment, IV insulin, acidosis or those undergoing a surgical procedure requiring anesthesia. More stringent acceptance criteria of TAE of 8.4% (based on inter- and intra-individual variability) and HI of 50 for baseline samples were also used. **Results**: Our initial analysis of over 500 matched specimens demonstrated that K levels may be successfully corrected in hemolyzed specimens with HI up to 400. However, significant number of outliers falling between 0.5 and 1.0 mmol/L was observed, suggesting that patients K levels may have changed between the baseline and test specimen collection due to treatment or other medical intervention. The analysis of a more stringently extracted patient subset where all the potential factors (intervention, treatment, etc) that may change K levels clinically were excluded, revealed that for 64 of 72 (89%) patient samples eK was within 8.3% of baseline K. It is also of note that for 96% (69/72) samples, eK value was within 0. 5 mmol/L of baseline and, in 65% (47/72) cases, eK value within 0.25 mmol/L of baseline. **Conclusions**: Accurate and timely estimation of potassium in the setting of hemolysis has a potential to significantly improve quality of patient care by reducing the specimen rejection rate and minimizing delay in necessary interventions. We have shown that by incorporating patient's own hematological parameters (Hgb and Hct), intra-cellular K contribution can be calculated and used to adjust measured K in the setting of in vitro hemolysis. Future studies include clinical validation of this equation on both critically ill and normal patient populations, using both serum and plasma specimens.

A-164

Method Comparison of Radiometer ABL90 FLEX versus ABL835 FLEX for Bilirubin in Arterial, Umbilical Cord, and Venous Whole Blood from Neonatal Subjects

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Background: Total bilirubin (tBil) is routinely measured in neonatal patients to monitor jaundice and guide clinical management. Blood gas analyzers with capability to measure tBil in whole blood offer an option to monitor tBil in neonates using low sample volume, which may be especially useful in neonatal intensive care units. An additional advantage may be conferred if non-laboratory personnel such as nurses and respiratory therapists can perform the analyses on analyzers close to patients. The objective of the study was to verify that the neonatal tBil measured on the Radiometer ABL90 FLEX analyzer (ctBil parameter) is a suitable replacement for current test method for arterial and venous whole blood samples, using the ABL835 FLEX as the predicate device. **Methods:** The study was performed at two academic medical centers in the United States with Institutional Review Board approvals. tBil was measured in arterial or venous whole blood by clinical laboratory technician or technologist on the ABL835 FLEX as part of routine clinical care. If there was sufficient residual specimen, non-laboratory personnel (e.g., nurse or respiratory therapist) measured tBil on the ABL90 FLEX. Spiked umbilical cord blood specimens were used for the remainder of the comparisons. **Results:** The table below shows the method comparisons between ABL835 FLEX (predicate) and ABL90 FLEX.

	Arterial	Venous	Cord (Spiked)
N	44	42	17
R	0.983	0.991	0.997
Equation	Y = 0.98x -0.54	Y = 0.98x -0.32	Y = 0.97x -0.58
Standard error	0.53	0.62	0.68
Range (mg/dL) - ABL90	1.7 - 13.6	1.6 - 28.1	1.8 - 37.3
Range (mg/dL) - ABL835	2.3 - 13.3	2.1 - 29.0	2.8 - 38.2

The ABL90 FLEX has limit of blank of 1.1 mg/dL, limit of detection of 1.6 mg/dL, and limit of quantitation of 1.6 mg/dL. The ABL90 FLEX requires 65 µl sample for tBil when used in syringe mode compared with approximately 95 µl in the ABL800 FLEX series. **Conclusion:** There was excellent correlation between ABL835 FLEX and ABL90 FLEX for measurement of tBil in neonatal patients across a wide range of tBil concentrations. ABL90 FLEX tBil measurements may be performed by non-laboratory personnel, providing more options for testing near patient locations.

A-165

Quantitation of Glycocholic Acid and Unconjugated bilirubin in Human Bile for Gall Bladder Diseases by Flow-injection MS/MS Using Standard Addition.

R. Kakarla[1], R. Voggu[1], J. Donaldson[2], B. Guo[1]. *[1]Cleveland state university, Cleveland, OH, [2]Mississippi State University, Starkville, MS*

Background:
Bile and its constituents are directly in contact with the biliary epithelium, making bile an ideal fluid for quantification. The emergence of endoscopic retrograde cholangiopancreatography has made sampling of bile possible without surgery. Recent findings reveal that changes in the levels of Glycocholic Acid (GCA) and Unconjugated Bilirubin (BLB) in bile are associated with Cholangiocarcinoma and Cholelithiasis respectively. Hence, we have developed the first quantification method for determination of GCA and BLB in human bile using dilute and shoot flow-injection MS/MS with standard addition to avoid column carry over and matrix effects.
Methods:
Bile samples were first diluted with methanol: DMSO (1: 3) and aliquots were used to prepare calibrators (12.5-200.0 ng/mL) by spiking GCA, BLB and internal standard for standard addition. The samples were then centrifuged and 10uL of supernatants were transferred to auto sampler vials. Flow Injection was performed by pumping 90% methanol into the ESI source of triple quadrupole tandem mass spectrometer by-passing the column compartment of HPLC at 0.3 mL/min for 2.5 minutes. Quantitation was done in negative MRM with mass transitions for GCA, BLB and IS set at 464.1-74, 583.6-285.3 and 401.2-249.1 respectively. Standard addition plots were made using peak area ratios to determine concentration of analytes in the samples.
Results:
We have developed a dilute-and-shoot FI-MS/MS method for the quantitation of GCA and BLB in human bile and applied it to clinical samples. Our method was validated according to the FDA guidelines. Additional transitions were monitored throughout the analysis for both GCA and BLB to ensure specificity. The method was found to be linear with a mean correlation coefficient of 0.99 for both GCA and BLB in the range of 12.5-200 ng/mL. The %RSD for the LLOQ was less than 15%. Accuracy, intra and inter-day precision were determined using three QCs at 31.25, 70.71 and 160.00 ng/mL. The %RE of intra, inter assays for GCA were 7.38-14.88, 9.52-14.80 and BLB were 0.09-8.67, 0.52-2.66 respectively. The %RSD of intra and inter-assays for GCA were less than 7.23 and 9.02. The %RSD of intra and inter-assays for BLB were less than 10.81 and 14.07. The absolute and relative matrix effects matrix effects of GCA were less than 9.72 and 12.6 respectively. The absolute and relative matrix effects matrix effects of BLB were less than 9.91 and 1.86 respectively.
Conclusion:
Our method is very advantageous in a clinical setting. First, there is no need for sample purification prior to analysis. Our method is sensitive even after 800,000 times dilution. Standard addition minimizes matrix effects caused by matrix components if at all present in diluted samples. Flow-injection eliminates the problem of column carryover which would otherwise require high solvent usage for the maintenance of a clean column. Third, the method is very fast with a run time of 2.5 min enabling high through put analyses of over 570 samples a day.

Tuesday, July 31, 2018

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

Endocrinology/Hormones

A-166

Testosterone levels evaluation in a cohort of 1 million Brazilian women.

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Background: Testosterone is the most important male hormone and it is present in women in low concentrations. Currently low dose supplementation of this hormone in women has been designated due to beneficial effects. However, the indiscriminate use and without medical supervision can cause risk of toxicity influenced by the route of administration, dose and individual sensitivity that lead to adverse effects. Although the use of androgens is controlled by prescription, access to these medicines has been increasing with unregistered formulas, herbal medicines, dietary supplements and illegal market. **Objective:** To evidence the alteration of testosterone level in clinical samples from women of different age groups during the period from 2013 to 2017. **Methods:** This is a study carried out through consultation of data collected of total testosterone test (Beckman Coulter) in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) database during the period of 2013 to 2017. The lower limit of reference range for males (175 ng/dL) was used to designate the results as altered. The women were stratified in groups by age (≤ 17 years; 18 to 45 years and ≥ 46 years). **Results:** A total of 942,625 women were evaluated from 2013 to 2017. The results showed that there was an increase in absolute number of altered results in age group of 18-45 years and a decrease in age group ≤ 17 and ≥ 46 years. **Conclusions:** Despite the lack of objective clinical data, and assuming stability in the prevalence/incidence of the main diseases associated with the elevation of testosterone in women, therefore, these results allow to infer that the possible supplementation of testosterone could be the responsible for this profile. These results help to better understand the profile of testosterone results released by the laboratory, as well as signaling to evaluate the current practices of hormonal supplementation in women.

Table 1: Absolute number of altered results in women.

YEARS	TOTAL NUMBER	ALTERED RESULTS	≤17 YEARS	18-45 YEARS	≥46
2013	99,593	2,180 (2.18%)	167 (7.7%)	853 (39.1%)	1,160 (53.2%)
2014	135,656	2,615 (1.92%)	171 (6.6%)	1,083 (41.4%)	1,360 (52.0%)
2015	163,531	3,575 (2.18%)	239 (6.7%)	1,596 (44.6%)	1,740 (48.7%)
2016	247,317	5,362 (2.16%)	300(5.6%)	2,764 (51.5%)	2,298 (42.9%)
2017	323,528	7,534 (2.32%)	357(4.7%)	4135 (54.9%)	3,042 (40.4%)

A-167

Evaluation of estradiol levels in patients using selective estrogen receptor modulators

J. F. F. Oliveira, T. C. R. A. Rosa, N. M. A. Rodrigues, L. A. Silva, A. L. Barbosa, L. F. Abdalla. *Laboratório Sabin, Brasília, Brazil*

Background: Selective estrogen receptor modulators (SERMs) are molecules that bind agonist or estrogen receptor antagonists (ERs) in specific tissues, which allow them different clinical performances. The action as an estrogen antagonist is used in the treatment of breast cancer. Recently, this type of employment has been approved in chemoprevention in women with high risk of developing breast cancer, because it acts in a competitive way to ERs, blocking the action of estrogen, which in its turn stimulates cell division in the breast. The purpose of this study is to evaluate the profile of estradiol levels in patients using SERM. **Methods**: The results are from a database of 85399 female patients – among the patients, 230 reported the use of SERMs Tamoxifen, Nolvadex and Faslodex. Concentrations of estradiol were determined by serum dosing that uses the chemiluminescence methodology. As cut-off point was used 356.7 pg/mL. **Results**: Of the 230 samples analyzed, 38 presented altered results. A percentage of 16.52% of patients that due to the mechanism of action of these drugs should have decreased results were found with increased results, because as these drugs have chemical structures similar to estradiol, there is cross reactivity possibility and inconsistent results referring to patients with clinical history. This observation confirms the importance of knowing the medication historic of the patient. **Conclusions:** Another interesting point of the study is that 26904 patients who did not report the use of medications and among these, 858 samples showed results above normal, which suggests the need for a more detailed evaluation of the person responsible for releasing the result, because this increase can be generated by the influence of medications as well as by other factors. Therefore, SERMs may contribute falsely increasing estradiol levels.

A-168

Indirect estimation of reference intervals from laboratory information systems for free thyroxin (FT4) and free triiodothyronine (FT3) in Ethiopian adults

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Aim

To establish indirect reference intervals for free thyroxin (FT4) and free triiodothyronine (FT3) for Ethiopian adults using data from laboratory information systems collected during routine laboratory activities as an alternative to resource-intensive population based methods.

Methods

All results for free thyroxin (FT4, n=7774 samples) and free triiodothyronine (FT3, n=7087) that were recorded in Ethiopia Public Health Institute, Clinical Chemistry Laboratory's laboratory information system between 2013 and 2016 were included in this study. Both FT4 and FT3 were measured using the Roche Cobas e 411 Clinical chemistry analyzer. We used the Reference Limit Estimator by Arzideh et al. to establish reference intervals, which estimates the proportion of samples from healthy individuals from a mixed population containing both pathological and physiological samples using a maximum-likelihood approach.

Results

We calculated combined reference intervals for males and females for FT3 (2.31-4.62 pg/ml) and FT4 (0.78-1.75 ng/ml).

Conclusion

Using laboratory information system data is an alternative method to validate and/or establish references intervals for low-and middle-income countries were laboratories often use kit insert references intervals, which are established in western countries and do not necessarily apply to the local population.

A-169

Performance Evaluation of the Atellica CH Enzymatic Hemoglobin A1c Assay*

J. Jones, C. Robinson, J. Gisiora, J. Dai. *Siemens Healthineers, Newark, DE*

Background: According to the World Health Organization, 422 million adults were living with diabetes globally in 2014, and an estimated 1.6 million deaths directly related to diabetes occurred in 2015. Eating a healthy diet, exercising regularly, and maintaining body weight are instrumental in delaying the onset of type 2 diabetes; early diagnosis is important for long-term diabetes care. One measurement of glycemic states is glycated hemoglobin (HbA1c). HbA1c is formed by a nonenzymatic Maillard reaction between glucose and the N-terminal valine of the β-chain of HbA, whereby a labile Schiff base is formed and converted into the more stable ketoamine (irreversible) via an Amadori rearrangement. A new enzymatic HbA1c Assay (A1c_E) has been developed* for the measurement of HbA1c on the Atellica® Solution Clinical Chemistry Analyzer. In the pretreatment step, the erythrocytes are lysed and the hemoglobin is oxidized to methemoglobin by reaction with sodium nitrite. In

the first step of the reaction (the Atellica A1c_E pack 1 + sample), the N-terminal fructosyl dipeptide fragment is cleaved from the hemoglobin beta chain with a protease. Concurrently, methemoglobin is converted into stable azidemethemoglobin in the presence of sodium azide, and the total hemoglobin concentration is determined by measuring the absorbance at 478/694 nm. In the second step of the reaction, fructosyl peptide oxidase (FPOX) is added to react with the fructosyl dipeptide to generate hydrogen peroxide. The hydrogen peroxide reacts with the chromagen in the presence of peroxidase to develop a color that is measured at 658/805 nm. **Methods:** Assay linearity was evaluated using Clinical and Laboratory Standards Institute (CLSI) protocol EP06-A. Precision was evaluated according to CLSI protocol EP15-A3. Two levels of a commercially available control and four whole blood pools ranging from ~4.50 to ~12.00% HbA1c were tested. Each sample was assayed five times per run, two runs per day, for 5 days. A method comparison study (n = 40 samples) was conducted between the Atellica CH A1c_E Assay and the National Glycohemoglobin Standardization Program (NGSP) secondary reference lab according to CLSI protocol EP09-A3. **Results:** The Atellica CH A1c_E Assay is linear from 3.80 to 14.00% HbA1c. Repeatability ranged from 0.29 to 0.65% CV, and within-lab precision ranged from 0.62 to 1.09% CV. The method comparison study yielded a regression equation of Atellica A1c_E Assay = 1.047 [NGSP] - 0.377% HbA1c (r = 1.00). The assay demonstrated a %TE ≤3.57 on the Atellica CH Analyzer. **Conclusions:** The A1c_E Assay on the Atellica CH Analyzer from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

A-170

Percentages of Hypo and Hyperthyroidism, findings of a large laboratory in the city of São Paulo.

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Background: Thyroid dysfunctions are very common in the world and primary care requires public policies with fast diagnostic and sensitive and specific tests as well as effective therapies for the treatment of their most prevalent disorders. From laboratory tests, thyroid stimulating hormone (TSH) dosing helps to diagnose a condition known as "subclinical hypothyroidism," which usually does not cause signs or symptoms, but hormone levels of TSH present at increased rates. The TSH test is also useful in the initial assessment of thyroid function and preferably use second or third generation assays that provide better diagnostic certainty over other traditional methods. TSH measurement is the most reliable test to diagnose the primary forms of hypothyroidism and hyperthyroidism, especially on an outpatient basis and in public health campaigns. Where possible, determination of the thyroxine free fraction (T4L) should be requested, since abnormalities in thyroid hormone-carrying proteins (secondary to the use of medications or certain clinical conditions) may alter the total concentration of T4 or T3. The ultra-sensitive TSH dosage (sensitivity 0.02 mIU / L) is the test of choice for the diagnosis of frank or subclinical hyperthyroidism. Excess thyroid hormones from any cause (except in rare cases of increased TSH) will result in suppressed TSH (usually <0.1 mIU / L); serum concentrations of free T4 will usually be elevated; in the absence of elevation of free T4 and presence of suppressed TSH, free T3 should be titrated (sometimes this is the first hormone to rise in both Graves' disease and toxic nodular goiter. OBJECTIVE-The authors aimed to analyze the results of TSH and T4L from a database of a large laboratory that serves several health units in Sao Paulo- Brazil. METHODS-The study was retrospective and observational for the period from 01/01/2012 to 12/31/2016, the results evaluated were patients of both sexes, above 18 years. The methodology used in the quantitative measurements of TSH and T4L in this period was a chemiluminescent assay, performed in an automated apparatus Architect i2000SR Immunoassay Analyzer - Abbott® Laboratories. RESULTS-The total number of requests in the period (2012 to 2016) was 4000299, respectively, 670326 (2012), 741418 (2013), 744082 (2014), 781664 (2015), 1062809 (2016).The analysis was based on calculating the percentages of the following situations: TSH and T4L, above / below the reference limits, adult TSH = 0.34 to 5.60 uIU/mL and T4L = 0.54 to 1.60 ng/dL, where for each situation analyzed we find the following percentages respectively in the years 2012, 2013, 2014, 2015 and 2016. Normal: 88.9%, 89.3%, 90.6%, 89.6%, 82.1%; Hyperthyroidism: 7.3%, 7.1%, 6.0%, 7.2%, 7.10%; Hypothyroidism: 2.6%, 1.9%, 1.7%, 1.5%, 9.90%. CONCLUSION-Based on the data found, we can say that even with the increasing number of exams done by the laboratory, the prevalence of altered hyperthyroidism data remained constant.Increased requests suggest greater interest resulting from public awareness and campaigns.

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Hemoglobin A1c analysis using uncentrifuged & centrifuged samples.

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Background: The measurement of hemoglobin A1c (HbA1c) and plasma glucose using JCA-BM6010/c analyzer can be performed in the same tube to reduce the number of sample tubes required. Therefore, the recommendation of specimen used is venous blood after centrifugation (800 g, 5 min). Occasionally, HbA1c measurement is requested without plasma glucose. The objective of this study is to compare HbA1c measurement using uncentrifuged and centrifuged samples analyzed by JCA-BM6010/c analyzer as well as compare with HbA1c analysis using Cobas c513 analyzer. **Methods:** We collected 215 patient samples that were sent to the Central Laboratory, Department of Clinical Pathology. HbA1c measurement was performed using three different methods: (i.)Roche Cobas c513 (uncentrifuged whole blood) (ii.) JCA-BM6010/c (centrifuged whole blood) (iii.)JCA-BM6010/c (uncentrifuged whole blood; 800g x 5 minute). Hemoglobin concentration analysis was performed using Sysmex XN-3000. Samples were divided into 5 subgroups according to the level of hemoglobin (Hb): (1) <7 g/dL; (2) 7-9.9 g/dL; (3) 10-12 g/dL in female or 10-13 g/dL in male; (4) 12-15 g/dL in female or 13-15 g/dL in male; (5) >15 g/dL **Result:** Median (IQR) of HbA1c value (%NGSP) were 6.2 (5.7-7.2), 6.4 (5.9-7.4), 6.3 (5.9-7.3)% in c513, centrifuged JCA-BM6010/c and uncentrifuged JCA-BM6010/c, respectively. (P <0.001). Using Passing-Bablok regression analysis, the comparison of HbA1c analysis between c513 and centrifuged JCA-BM6010/c yielded a slope of 1.00 (CI 0.98 to 1.00) and intercept of 0.20 (CI 0.20 to 0.35). The comparison of HbA1c analysis between c513 and uncentrifuged JCA-BM6010/c yielded a slope of 0.50 (CI 0.36 to 0.63) and intercept of 0.94 (CI0.92 to 0.96). The comparison of HbA1c analysis between centrifuged and uncentrifuged samples using JCA-BM6010/c yielded a slope of 0.16 (CI -0.10 to 0.30) and intercept of 0.96 (CI 0.94 to 1.00). **Conclusion:** Both centrifuged and uncentrifuged samples gave comparable results in the analysis of HbA1c using JCA-BM6010/c analyzer. However, HbA1c analysis using JCA-BM6010/c showed systematic difference (centrifuged samples) as well as systematic and proportional differences (uncentrifuged samples) when compared with HbA1c analysed by c513 analyzer.

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Correlation of Thyroid Function with Biochemical Parameters And Baseline Characteristics in Obese Individuals

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Background: The prevalence of obesity is increasing worldwide and at the same time, the understanding of its pathogenesis and metabolic consequences is markedly advancing. Thyroid hormones (TH) play a key role in regulating energy homeostasis. Obesity has driven new interest in the relationship between thyroid hormone and weight status. **Aim:** To know the correlation of thyroid function with biochemical and baseline characteristics in obese subjects. **Method and materials:** This is a hospital based case-control study. There were 77 obese subjects (30 male and 47 female) and 50 controls (14 male and 36 female) with age and sex matched. Five ml of the venous blood was collected and kept in 12''x75'' gel tubes. Serum samples were used for biochemical parameters using (Erba Mannheim) XL- 300 Chemistry auto analyzer and thyroid function tests in CLIA. Waist and hip circumference were measured using a measuring tape. Statistical Analysis was done using SPSS version 17. Comparisons of mean values between controls and cases were done using students't' test. Pearson's bivariate correlation analysis was used to correlate variables between the controls and cases. $p < 0.05$ was considered to be statistically significant. **Results:** The age of control and obese subject (mean±SD)were33.08±11.02 and 35.88±9.37 respectively. Demographic parameter BMI, W/H Ratios and Waist circumference (mean±SD) in control and obese subjects were 21.90 ± 1.39, 0.86±0.08, 76.00±6.94 and 31.50 ± 5.09, 0.94±0.07, 96.24±17.34 respectively. The thyroid function test and biochemical parameters in control and obese subjects FT3, FT4, TSH, FBS, TC, TG, LDL-C were insignificant and HDL-C was significant. In this study, 5% obese subjects were having a thyroid disorder, out of which 75% were of subclinical hypothyroidism and 25% with primary hypothyroidism. Pearson correlation analysis between serum FT3, FT4, and TSH with respect to baseline characteristics of the study subjects reveal positive significant correlation ($p<0.05$) between FT4

with WHR in obese subjects and insignificant correlation (p>0.05) between other baseline characteristics. Similarly, Pearson correlation analysis between serum FT3, FT4 and TSH with respect to biochemical parameters of the study subjects showed positive significant correlation (p<0.05) between FT4 with TC and LDL-C in obese subjects and insignificant correlation (p>0.05) between other biochemical parameters. **Conclusion:** Our results showed thyroid disorder in an obese subject is hypothyroidism and FT4 have a positive significant correlation with waist/hip ratio, TC, and LDL-C. **Key words:** Thyroid Hormones, Waist and Hip Ratios, Obese**,** Baseline characteristics

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Assessment of The Effects of Gestational Diabetes on Some Anthropometric Indices.

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Background: Gestational diabetes mellitus (GDM), a type of DM is a condition in which women develop DM during pregnancy. GDM is a threat to pregnant women because of its short and long term risks for them and their neonates. Ability to properly prevent or manage this state depends on identification of markers. This study was designed to access the effect of gestational diabetes on blood glucose and some anthropometric markers. **Methods:** The research subjects were selected from people in Ado-Ekiti metropolis of Ekiti State, Nigeria. Group 1 was made up of fifty (50) gestational diabetic women (GDM); group 2 fifty (50) normal pregnant women (NP); group 3 fifty 50 diabetic non-pregnant women (DM) and group 4 was made up of 50 non-diabetic non-pregnant women (ND-NP). Data for anthropometric measurement and blood fasting sugar were determined using standard methods. The data collected from the results were analysed using one - way Analysis of Variance (ANOVA) followed by post-hoc Duncan test, and expressed as mean ± standard deviation(SD) with P value less than 0.05 (p<0.05) considered to be statistically significant. **Results:** The results showed increased BMI in kgm^{-2} in GDM (32.38±4.25) and diabetic women (DM) (31.95±12.48) compared to normal pregnant (27.85±8.58) and non-diabetic non-pregnant women (25.24±3.30). The gestational age (in weeks) of GDM (17.76±5.46) and normal pregnant (NP) women (17.62±3.33) showed no significant difference (p>0.05). There was also a significant increase (p<0.05) in FBS (mmol/l) of GDM (6.10±1.49) and diabetic patient (12.16±6.86) compared to normal pregnant (3.74±0.66) to non-diabetic non-pregnant women (4.23±0.60). **Conclusion:** It can be concluded from this study that monitoring anthropometric indices in the gestational period may serve as a means of detecting and managing gestational DM

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Investigating Macroprolactin in a tertiary care hospital

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Background: Prolactin (PRL) circulates as a heterogeneous mix of monomeric PRL (85%) and larger molecular weight forms termed macroprolactin (macroPRL). MacroPRL is under-recognised and may cause mis-diagnosis, unnecessary investigation and inappropriate treatment of hyper-prolactinemia. The prevalence of macroPRL in hyper-prolactinemia has been variously reported as between 4.0 to 46%. All available PRL assays cross-react with macroPRL. Re-assay of such sera after polyethylene glycol (PEG) precipitation to deplete macroPRL is advised but not universally implemented in all laboratories . We investigated the prevalence of macroPRL in our immunoassay section in a 1000-bed tertiary care teaching hospital. Methods: Prolactin is performed on the Abbott Architect i2000SR immunoassay analyzer in our department. Patient requests for serum prolactin (n = 616) were studied over a 9 month period. Consecutive samples with PRL >600 mIU/L (n=100) were stored at -20°C and re-tested after treatment with equal volume of 25% PEG6000 at room temperature for 10 mins. Following centrifugation (20000g for 2 minutes) PRL was measured in the supernatant. Samples with PEG-precipitated PRL of <40%, 40-60%, >60% were considered as negative, borderline or positive for macroPRL respectively. We also compared the PRL data with those performed similarly on another immunoassay platform (Roche Cobas e602 analyzer) . Results: Pre-PEG PRL ranged from 605-18326 mIU/L (median 1178) for Architect PRL and 316-24159 (median 1289) for Cobas PRL. The post-PEG PRL ranged from 91-14346 mIU/L (median 742)) for Architect PRL and 105-19336 (median 1608) for Cobas PRL.. The Architect PRL identified 18 subjects (13 men) as macroPRL, 11 borderline and 71 negative while the Cobas PRL classified 12 patients (8 men) as macroPRL, 5 borderline and 83 as negative. For the Architect macroPRL pre-PEG PRL ranged from 635-1523 mIU/L and declined to 91-356 mIU/L after PEG treatment while the corresponding vales for Cobas macroPRL was 401-1327 mIU/L and 105-462 mIU/L respectively. All 12 macroPRL classified by Cobas were also identified as such by Architect. Passing-Bablok regression analyses showed closer agreement between Architect and Cobas post-PEG PRL values (regression equation: Cobas = 1.373108 Architect + 0.149142, Spearman correlation coefficient of 0.972) than pre-PEG PRL values (regression equation: Cobas = 1.399187 Architect - 197.355195, Spearman correlation coefficient of 0.899). Conclusion: There was greater impact of macroPRL on hyper-prolactinemia with the Architect assay than the Cobas. However, there was 100% concordance between Cobas identified macroPRL and Architect macroPRL. The preliminary prevalence of macroPRL in our study (18% with Architect PRL and 12% with Cobas PRL) is not inconsequential. It is prudent for clinical laboratories to provide value and accurate results. A reflex investigation for macroPRL in all cases of hyper-prolactinemia is such an initiative.

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Vitamin-D diminishes high platelet aggregation found in patients with Type 2 Diabetes Mellitus

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Background: Type 2 diabetes mellitus (T2DM) is associated with increased risk for atherosclerotic diseases. Platelet activation is found in inflammatory conditions and implicated in the pathogenesis of T2DM and atherosclerosis, which are also associated with Vitamin-D deficiency. The aim of this study was to investigate the relation between platelet aggregation, Vitamin-D and HbA1c among healthy individuals and those with T2DM. The direct effect of Vitamin-D 1-25 (calcitriol) on platelet aggregation was also investigated. **Methods:** Platelet aggregation was examined with and without calcitriol pre-treatment, using collagen or adenosine diphosphate (ADP) as agonists in study groups: A. normoglycemic: HbA1c<5.7; B. Pre-DM: 5.7%≥HbA1c≤6.4%; C. DM and aspirin therapy: HbA1c>6.4(+)Asp.; and D. DM not on aspirin therapy: HbA1c>6.4(-)Asp. **Results:** Platelet aggregation was higher in DM(-)Asp compared to normoglycemic and DM(+)Asp, and higher, but not significant compared to pre-DM. The study population exhibited negative correlation between HbA1c and Vitamin-D25 serum concentration. Excluding DM(+)Asp, aggregation induced by collagen was significantly higher in patients with insufficient (<76nmol/L) Vitamin-D25 compared to sufficient (≥76nmol/L) Vitamin-D25. Negative correlation was found between Vitamin-D25 serum concentrations and collagen-induced aggregation. In the DM(-)Asp, collagen-induced aggregation decreased after calcitriol treatment. Calcitriol reduced ADP-induced aggregation in control and DM(+)Asp groups. **Conclusion:** High platelet aggregation is associated with high HbA1c and low Vitamin-D25 levels. This elevated aggregation could be regulated by a novel, direct effect of calcitriol, indicating a beneficial effect of Vitamin-D on atherosclerosis and on vascular complications related to diabetes. We suggest a non-genomic mechanism for the Vitamin-D/Vitamin-D receptor (VDR) pathway.

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Polymorphisms of LEP, LEPR, DRD2, HTR2A and HTR2C genes and risperidone- or clozapine-induced hyperglycemia

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Background: To determine whether the genetic polymorphisms, LEP promoter 2548G/A (*LEP*), LEPR c.668 (*LEPR*), dopamine D2 Tag-SNP (*DRD2*), serotonin 5-HT2A (*HTR2A*) and 5-HT2C (*HTR2C*), associate with risperidone- or clozapine-induced hyperglycemia in Thai adult psychotic patients. **Methods**: In this cross-sectional analysis, blood samples were obtained from 180 Thai psychotic patients treated with a risperidone (n=130) or clozapine-based (n=50) regimen. Blood samples were genotyped for the above-mentioned polymorphisms by using the TaqMan assay (Roche Diagnostics, USA); they were also analyzed for glucose, lipid profile; i.e. total cholesterol, triglycerides, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol, and hormones; i.e. adiponectin, leptin, insulin and prolactin. Differences among groups were analyzed using the χ^2 test, Mann-Whitney U test or *t* test where appropriate. To determine the associations between the genetic factors as well as clinical risk factors with the hy-

perglycemia, a backward, stepwise multivariable logistic regression model was used. **Results:** The prevalence of hyperglycemia was greater among patients receiving clozapine (64.0%) than risperidone (30.8%). Metabolic biomarker results were similar in the two subject groups, except that the clozapine group showed higher fasting glucose and lower prolactin. Among candidate genes, only *LEP* 2548G/A polymorphism demonstrated significant association with the hyperglycemia ($\chi^2 = 9.879$, $p = 0.008$) in risperidone-treated patients; those with AA genotype had the highest risk (41.1%), followed by AG (20.1%) and GG (0%) genotypes. Among clozapine-treated patients, the study genes and hyperglycemia were not associated. Using binary logistic regression, *LEP* 2548G/A gene demonstrated the significant association with hyperglycemia, independent of BMI in patients on risperidone; the odds ratio (95% confident interval) was 0.314 (0.138-0.715), $p = 0.006$. By contrast, none of the polymorphisms, except for BMI significantly associated with hyperglycemia in patients on clozapine. **Conclusions:** The risk of hyperglycemia was associated with *LEP* 2548G/A polymorphisms among Thai adults receiving risperidone, but not those receiving clozapine. Polymorphism of *LEP* 2548G/A may affect the risperidone-induced glycemic dysregulation in Thai patients. The other polymorphisms under study did not appear to have any impact on the risk of hyperglycemia. Understanding the mechanisms and risks for hyperglycemia provides an opportunity to prevent impaired glucose metabolism in patients taking risperidone or clozapine.

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Derivation of truer metrics of long term patient variation of three contemporary hemoglobin A1c assays demonstrates both borderline and highly acceptable analytical performance

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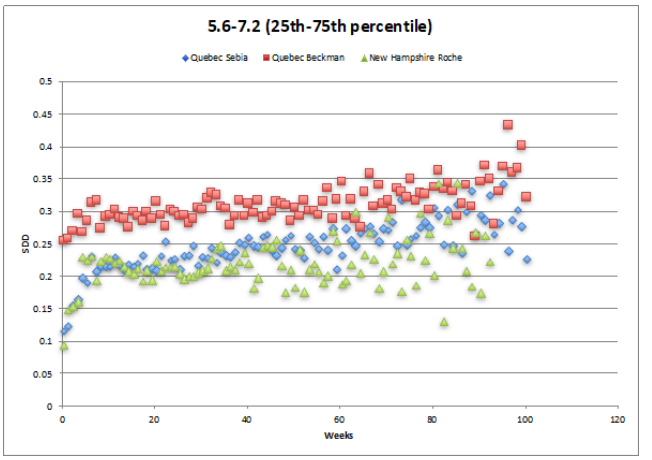
Introduction: Previously, we demonstrated that the statistical analysis of sequential intra-patient data can yield realistic measures of patient biologic and analytic variation. We have refined this analysis to determine long term (LT) intra-patient variation. To accomplish this, we determined all possible intrapatient pairs reported by the laboratory for several years and sorted these pairs by time between their sequential assays. The standard deviation of duplicates (SDD) was determined and charted for each time interval. We apply this analysis to three A1c assays. **Methods and Materials:** Patient HbA1c data were obtained from a Quebec laboratory replacing it's Beckman Coulter Synchron DxC®immunoassay (35,000 A1c from 15,000 patients) with Capillarys 2 Flex Piercing® (C2FP), (40,000, from 19,000 patients) and a New Hampshire laboratory operating the Tinia Quant Gen III, Cobas 8000, c502. AND Cobas 6000, c501 (121,000 HbA1c from 53,000 patients). We generated graphs of the LT intra-patient SDD of the individual methods for 3 patient subpopulations: low normal HbA1c, adequate glycemic control and poor control. **Results:** The Figure shows the LT SDD for the 25th to 75th percentile. The Beckman assay demonstrates the highest variation which is not evident in the Sebia assay which overlaps the Roche SDD. For the graphs of the other two populations, the low normal and the poor diabetes control patients, the SDDs overlap. **Discussion:** Essentially, the same patient population was sampled and assayed with the Beckman and Sebia assays. The Beckman assay obviously exhibits excess analytic variation. Sources of this variation include between instrument and between reagent lot variation. The magnitude of this increase in variation is roughly 0.1 divided by 6.5 or about 1.5%. For decades, we have maintained that the CV of HbA1c should be 2 to 3%. Future evaluations of the performance of HbA1c assays should include LT estimates of variation derived from stratified patient data.



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Serum Testosterone as a Severity Marker among Patients with Coronary Artery Disease

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Background: Male sex has been considered as an independent risk factor for cardiovascular disease (CVD) since many years. But recent studies have shown controversial results. The purpose of this study is to know the relation between testosterone and degree of severity of coronary artery stenosis in men diagnosed with coronary artery disease (CAD) via angiography. **Methods:** In this cross sectional observational study, 102 men were grouped into three categories according to the testosterone level tertiles. The inclusion criteria were male patients with CAD (angiographically proven), admitted in general ward of Manmohan Cardiothoracic Vascular and Transplant Center, Nepal, who provided written consent for the study. The exclusion criteria were- Patient refusal to participate in the study; Patients on any medication affecting sex hormone level like anticonvulsant and antithyroid drugs; Patient with carcinoma of prostrate or prostatectomy; Patient with major organ failure (respiratory/renal/liver); History of recent surgery or major trauma (within 3 months); Previous angioplasty.A brief medical history and morning fasting sample were obtained from each patient and blood sugar, total testosterone (TT) and lipid profile, SHBG were measured. Blood sugar and lipid profile were by using fully automated analyzer, BT 3000, Italy. Total testosterone (TT) was measured by enhanced chemiluminescent immunoanalyzer (ECI) and sex hormone binding globulin (SHBG) by ELISA kit. Free testosterone (FT) and bioavailable testosterone (BT) were calculated and for severity of coronary stenosis gensini score was used. The relationships were assessed using chi-square test, one way analysis of variance (ANOVA) and Pearson's Correlation. **Results:** Of the total 102 patients (mean age 62 years), majority of them (41.2%) had triple vessel disease.TT, SHBG, FT and BT were 346.1 ± 176.6 ng/dl, 44.5 ± 21.7 nmol/L, 0.2 ± 0.2 nmol/L and 5.0 ± 3.5 nmol/L respectively. Various CVD risk factors had no significant correlation with testosterone. Though negatively correlated, no significant association was found between gensini score and FT and BT (r=-0.054, p-value = 0.590 and r = -0.051, p-value = 0.617 respectively). Similar results were obtained when number of vessels involved and TT, FT and BT were compared. However, the number of diabetic patients gradually decreased with the increasing value of TT in the three tertile group (p-value = 0.040). **Conclusion:** Our study suggests that low testosterone is associated with risk of diabetes mellitus. However, it cannot strongly agree or disagree with negative relation between TT and CAD, and thus warrants further investigations which may include but not limited to use of measured value of FT and BT.

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Immunoassay harmonization (or the lack of it). The PTH assay example.

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Background: Capture and signal antibodies and standard/calibrator characterize immunometric assay design. Whatever combination, for harmonized assays, it is expected that different reagents give comparable results for the same patient sample. Two key components for immunoassay harmonization are the standard/reference system (that should be referred to an international reference preparation, if there is one), and antibodies combination that can recognize different epitopes. Harmonization, or lack of it, is visualized in external quality assessment schemes (EQAS). A clear example of this issue is plasma PTH measurement. Intact parathyroid hormone (PTH) is an 84-amino acid peptide, being the N terminal residue biologically active. Both plasma PTH 1-84 and PTH 1-34 disappear in few minutes. The C-terminal portion is more stable, particularly in patients with chronic renal failure (CRF), one of the clinical conditions in which PTH measurement is required. There are some other related proteins too, as PTH 7-84 and smaller fragments. Between-method differences are observed in EQAS, patients´ results show similar differences. **Methods:** To analyse the possible reasons of this lack of harmonization, components of the most used PTH intact and 1-84 PTH assays in the EQAS Buenos Aires (ProgBA) were compared. Data were taken from inserts provided by each IVD manufacturer. **Results:** a) *PTH reference preparations:* ABBOTT 79/500; CENTAUR 79/500 (73 % mean WHO standard recovery); ROCHE 95/646; IMMULITE (traceable to an internal standard); LIAISON not stated; BECKMAN 79/500 (average WHO recovery 57 % and 53 % for Routine and Intraoperative Modes). *b) Immunoassay design:* Capture and signal antibodies were different for each company, their selectivity was to peptides 1-84, 1-34, 1-37, 38-84, 39-84, 44-84, depending IVD manufacturer. ROCHE and LIAISON developed assays that claim to recognize the complete molecule, 1-84. Different selectivity against PTH fragment 7-84 was stated: 48.3 % for IMMULITE, 72 % for BECKMAN, 52 % LIAISON intact, LIAISON 1-84 0%, others not stated *c) EQAS results:* for a sample from CRF patients, medians in pg/mL were: ARCHITECT: 606, BECKMAN: 350, CENTAUR: 436, IMMULITE: 484, ROCHE INTACT: 356, LIAISON 1-84: 166, ROCHE: 1-84 187. The standards utilized by different IVD manufacturers are not the same, there are two reference preparations, 79/500 and 95/646, with even different assay recovery. Some methods do not state calibration to an IRP. When immunometric assay design is analyzed, it can be noticed that selectivity of antibodies used is quite different for each kit, detecting related peptides in different proportions. 1-84 methods give lower results as expected, but they don´t seem to be harmonized. These differences are shown in EQAS results, stressing the importance of EQAS in methodologies´ follow-up. **Conclusion:** If immunometric assays are calibrated against different preparations IRPs, if available, and antibodies in immunoassay designs recognize different fragments, active or not, it is impossible to achieve harmonization. In order to produce clinically useful and comparable patient results it is crucial that IVD industry agrees in selectivity and calibration to expand the traceability chain to higher order.

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Evaluation Of Beta hydroxybutyrate (STANBIO Laboratory) Reagent On Beckman Coulter AU5800 Chemistry Automated Analyser

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Introduction
Ketosis is a common feature in acutely ill patient, patient with acute alcohol abuse, Diabetes Mellitus and starvation. Measurement of β-hydroxybutyrate (BOHB) rapidly and reliably is critical for diagnosis and management of ketosis crisis, hence we evaluated and compared 2 different BOHB kits on the Beckman Coulter AU5822 automated platform to determine their ease of use and turn-around-time (TAT)
Method
STANBIO Laboratory β-hydroxybutyrate LiquiColor reagent (STANBIO) and RANBUT (Randox Laboratories) reagent are both an end-point enzymatic assay. They were evaluated for correlation, precision, linearity, detection of limit and dilution verification (performed on-board of analyser).
Results
26 patient samples with concentration ranging from 0.15 mmol/L to 2.85 mmol/L correlates well with y=1.0183x + 0.0386, r = 0.9973. Absolute difference varied between -0.10 mmol/L to 0.22 mmol/L with percentage difference between -14.3% to 22.2 %. Slightly positive bias was observed across analytical range (up to 3.20 mmol/L) especially at the lower concentration. At lower concentration from 0.15 mmol/L to 0.45 mmol/L, correlation is y = 1.1053x-0.0041, r = 0.986, n = 11. Due to differences in upper analytical range (STANBIO at 4.50 mmol/L as compare 3.20 mmol/L for RANBUT), 6 additional samples were included. Correlation fairs even better with y = 1.0057x - 0.0032, r = 0.9993, n = 32 for BOHB concentration up to 4.10 mmol/L. Absolute difference ranges from -0.01 mmol/L to 0.22 mmol/L with percentage difference -3.2% to 8.3%. Total imprecision was 0.3% to 3.0% CV for concentrations ranging from 0.17 mmol/L to 3.52 mmol/L whilst higher CV of 1.6% to 3.4% for concentration between 0.26 mmol/L to 2.99 mmol/L for RANBUT. Linearity is within ± 10.0% for concentration between 0.18 mmol/L to 4.15 mmol/L while RANBUT fairs better with ± 5.0% between 0.25 mmol/L to 2.86 mmol/L. Lower detection limit for both STANBIO and RANBUT is the same at 0.02 mmol/L. With AU5800 onboard auto-dilution 1:2 ratio using deionized water shows 95.2 % to 102.7 % recovery with BOHB concentration up to 6.81 mmol/L and similarly RANBUT reagent recovers 92.6 % to 107.9 % with concentration up to 3.70 mmol/L. Assay time for both reagents is 10 minutes and average TAT is between 30 minutes with Laboratory Automated System. With RANBUT reagent, on average up to 15% of patient samples requires further dilution. Occasionally, assay exhibits kinetic error due to reaction instability although BOHB concentration is well within claimed analytical range. Up to 60 minutes is need for either a neat sample re-run or further automated and sometimes manual dilution. These additional interventions compromised the desired TAT significantly. However, these phenomena were not seen with STANBIO reagent.
Conclusion
STANBIO assay demonstrates good analytical performance and precision on Beckman Coulter AU5800 analyser. In summary, STANBIO reagent is more suitable for automated instrument design for Laboratory Automated System for fast turn- around-time resulting

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Are the immunoturbidimetry and HPLC techniques interchangeable in the determination of glycosilated hemoglobin?

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Background: Diabetes mellitus (DM) is a disease characterized by an alteration in the metabolism of carbohydrates, defined by chronic hyperglycemia. HbA1c is a commonly used tool for the management and adjusting the treatment of diabetic patient's. The American Diabetes Association (ADA), the European Association for the Study of Diabetes and the International Diabetes Federation, after reviewing existing evidence, recommend it as a diagnostic test for DM when its values are greater than or equal to 6.5%.
Methods: The aim of this study is to compare 2 automated methods to measure HbA1c based on different principles of measurement HPLC (AKRAY HA 8180V, Menarini Diagnostics) and immunoturbidimetry (Tina-quant Hemoglobin A1c Gen.3, Cobas 311, Roche Diagnostics), evaluating the correlation Between both. We worked by verifying the correct quality requirements for both measuring instruments. 450 samples of whole blood (EDTA) were analyzed by both analytical systems
Results: HbA1c values were obtained between 4.4% and 13.3% (median = 6.4%) by HPLC and between 4.5% and 12.4% (median = 6.4%) by immunoturbidimetry. The Rho correlation coefficient of Spearman was 0.99 ($p < 0.0001$). Using the Bland and Altman test, we obtained an average of the differences between both methods of 0.04% and the regression of Passing and Bablot was HPLC = 0 + 1 x Immunoturbidimetry. These results corroborate results obtained previously in our laboratory with a smaller number of samples
Conclusion: It should be considered that changes will be made in the therapeutic regimens guided by the HbA1c level of the patient and the sequential changes of their measurements, whether or not they know the analytical performance of the method: adequate or not. This condition that undoubtedly, must be ensured by the biochemical professional. The clinical laboratory has a great responsibility in the choice of the analytical method to quantify HbA1c before the wide range of methodological possibilities offered by the in vitro diagnosis. Although the complexity of the laboratory is one of the factor that will influence this choice, it's neccesary to ensure the use of reliable, high quality tests that meet the stipulated analytical requirements, because it will directly impact the quality and clinical utility of the laboratory. result issued. At the beginning, the determination of HbA1c showed great variability between the different methods and laboratories. Currently, analytical methods can be considered "interchangeable" The correlation between both methods of measurement was very high and the average of the differences between both methods was negligible. Both methods can be interchangeable. The HPLC includes the chromatogram, with which most of the variant hemoglobins can be separated and identified, and can even show silent hemoglobinopathies. However it is an instrument of exclusive use, and other

determinations can not be made simultaneously. Acording with the bibliography the advantages of the immunoturbidimetric method are optimization of processing time of HbA1c tests and a reduction in the unit cost per test. Although HPLC is the reference technique, immunoturbidimetry is a reliable method for measuring HBA1c.

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Effect of Sample Storage Conditions on Vitamin D Metabolites

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Background: Methods for measuring various vitamin D metabolites are increasingly used to investigate vitamin D metabolism and its clinical associations. However, the stability of these newly measured metabolites are not well known therefore we aimed to determine the stability of vitamin D metabolites, namely 25(OH)D_3, 1,25$(OH)_2D_3$, 24R,25$(OH)_2D_3$, 25(OH)D2, and 3-epi-25(OH)D_3 in plasma samples under different standing/storage conditions. **Methods:** Blood specimens were collected from volunteers (n=20) into K2EDTA tubes (Becton Dickinson, NJ, USA), centrifugated, aliquoted, and analyzed immediately to represent baseline values. Samples were kept at +25°C and +4ºC for short-term storage (24 and 72 hours), -20ºC for mid/long-term storage (10 and 30 days) and -80ºC for long-term storage (30 days), all in dark and analyzed with high-performance liquid chromatography tandem mass spectrometry (LC/MSMS-8050, Shimadzu Co., Kyoto, Japan). Additionally, a group of derivatized extracts of the samples were kept at +4ºC and analyzed on the 5th day, to determine on-board stability. The results obtained under different conditions were compared to baseline values and relative bias percentages (RBP) were calculated. Medians of the RBP of each group were compared to calculated acceptable change limits (ACL=$\sqrt{2}\cdot Z\cdot$CVa; Z=1.96 which is determined by the 95% confidence interval value, Cva is the analytical imprecision calculated from quality control materials at concentrations similar to median analyte concentrations of volunteers). **Results:** The RBP of all metabolites did not exceed the ACL values in any of the tested groups. Plasma samples were found to be stable under the tested durations and temperatures (Table 1). Table 1. Effect of sample storage conditions on vitamin D metabolites (CVa, analytical imprecision; ACL, acceptable change limits; RBP, medians of the relative bias percentages; * derivatized samples)

Analytes	Median	CVa (%)	ACL (%)	RBP (%)+ 25°C, 24h	RBP (%)+ 25°C, 72h	RBP (%)+ 4°C, 24h	RBP (%)+ 4°C, 72h	RBP (%)- 20°C, 10d	RBP (%)- 20°C, 30d	RBP (%)- 80°C, 30d	*RBP (%)+ 4°C, 5d
25 (OH) D_3	11,0 ng/mL	9,3	25,8	11,8	2,8	-0,9	8,1	4,1	3,7	-4,4	-0,4
1,25 $(OH)_2$ D_3	33,1 pg/mL	9,1	25,2	4,4	6,1	-19,1	12,7	-15,6	10,9	-2,4	9,4
24R,25 $(OH)_2$ D_3	0,6 ng/mL	7,6	21,1	-1,2	5,7	5,0	12,5	5,9	-7,0	-7,2	11,0
25 (OH) D_2	0,4 ng/mL	5,8	16,1	-4,2	-0,1	-1,9	11,3	-1,4	-4,6	-8,0	11,8
3-Epi-25 (OH) D_3	0,5 ng/mL	7,4	20,5	9,1	0,5	-6,1	-3,4	-5,4	-3,6	-8,7	-18,6

Conclusion: Blood samples for vitamins D analyses or derivatized extracts can be processed under the tested laboratory conditions.

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A Method for Depleting Thyroglobulin from Human Serum for Use in Performance Monitoring of an In Vitro Diagnostic Assay

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OBJECTIVE: Clinically relevant performance monitoring of *In Vitro* Diagnostic (IVD) assays during the development and/or manufacturing process is best achieved using specimens from human sources containing native analyte at medically relevant concentrations. Human Thyroglobulin (Tg) exists in circulation as a 660 kDa, dimeric protein, produced by the follicular cells of the thyroid and is the precursor of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Monomeric subunits can be found in circulation as well and may react with some immunoassays. Tg concentrations in normal human serum can be 10 to 20-fold higher than those observed in patients that have undergone full or partial ablation of the thyroid gland. In these patients, the Tg levels usually are very low or negative. As a result, it is necessary to deplete this analyte in an effort to create samples for use in measuring assay performance while concurrently maintaining matrix integrity. In this present study, we describe a novel method for depleting Tg from normal human serum was evaluated. **METHODS**: To maintain matrix integrity, the mass of Tg was exploited by employing size exclusion tangential flow filtration via a 300 kDa cutoff membrane. This method was designed to deplete monomeric and dimeric Tg while retaining serum proteins having a mass less than 300 kDa, e.g. immunoglobulins and albumin. **RESULTS:** A 2.2 liter pool of human serum was processed using a Millipore Pellicon-2 Mini housing equipped with a Millipore Biomax 300 kDa cutoff cassette. The apparatus was operated in accordance with the manufacturer's instructions, and the filtrate was collected. Initial and post-filtration Tg concentrations were measured using a research phase IVD assay and determined to be 20.24 ng/mL (n=1) and 0.00 ng/mL (n=10) respectively. In theory, the anticipated process efficiency for depletion should be >99%. **CONCLUSIONS:** Size exclusion tangential flow filtration is an effective means of non-specifically reducing analytes of interest from human serum.

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Performance Evaluation of Immunoassays on the Atellica IM 1600 Analyzer at Friarage Hospital

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Background: At Friarage Hospital, studies were performed to assess the analytical performance of immunoassays (IM) for the Atellica® IM 1600 Analyzer with respect to verification of precision, linearity, and method comparison with Siemens Healthineers assays on the ADVIA Centaur® XP/XPT System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using approximately 44 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material ranged up to nine depending on the assay; for each assay, three replicates of each sample level were assayed. **Results:** Overall within-run and total imprecision agreed with the manufacturer's claims. Within-run IM CVs ranged from 0.0% to 7.9% and total IM CVs from 1.3% to 14.6%. Linearity studies were performed for all assays. Precision and method comparison studies are summarized below.

		Precision			Method Comparison
Atellica IM Analyzer Assay	**Units**	**Mean conc. (low, high)**	**Within run %CV(SD) (low, high)**	**Total %CV(SD) (low, high)**	**Atellica IM Analyzer vs. ADVIA Centaur XP/ XPT System**
Fer	pmol/L	14.66, 310.32	2.7(0.40), 3.6(11.27)	3.9(0.57), 5.4(16.63)	*
VB12	ng/L	173.84, 722.24	7.9(13.71), 2.9(21.05)	14.6(25.3), 7.5(54.19)	*
VitD	nmol/L	72.81, 245.87	5.1(3.75), 2.6(6.40	7.6(5,51), 3.6(8.87)	*
iPTH	pg/mL	3.99, 91.05	2.0(0.08), 1.5(1.36)	2.1(0.09), 1.7(1.52	*
BNP	ng/L	43.00, 466.08	2.8(1.19), 1.4(25.51)	2.9(1.23) 2.2(39.25)	*
PSA†	ng/mL	0.14, 16.03	3.5(0.00), 1.9(0.31)	6.0(0.01), 2.4(0.39)	y=1.02x-0.03(XP)
AFP†	IU/mL	29.24, 223.77	3.7(1.10), 2.4(5.26)	4.4(1.29), 4.2(9.37)	*
CEA	u/L	2.53, 38.85	4.7(0.12), 2.2(0.84)	6.4(0.16), 2.5(0.98)	*
eE2	pmol/L	139.40, 3594.36	3.7(5.22), 2.4(85.04)	6.6(9.14), 3.5(124.1)	y=0.99x-34.3(XPT)
ThCG†	mIU/ mL	5.71, 395.38	5.5(0.31), 2.2(8.63)	6.6(0.37), 2.4(9.34)	*
PRGE	nmol/L	3.69, 68.89	4.9(0.18), 2.7(1.85)	7.5(0.28), 3.2(2.20)	y=0.999x-0.47(XPT)
TSTII	nmol/L	0.72, 43.23	2.9(0.02), 6.2(2.70)	5.3(0.04), 8.1(3.50)	y=0.98x+0.14
TSH3UL	uIU/ mL	0.01, 29.69	0.0(0.00), 1.3(0.39)	0.0(0.00), 1.3(0.40)	*

*Comparison not completed at this time. **Conclusions:** Overall the assays tested on the Atellica IM 1600 Analyzer demonstrated good precision and correlation to the ADVIA Centaur XP/XPT System assays. Generally, the precision results were consistent with manufacturer's claims. HKD also at nstitute of Cellular Medicine, Newcastle University, United Kingdom. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. † Not available for sale in the U.S. Future availability cannot be guaranteed.

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Comparison of Sample Preparation Options for the Extraction of a Panel of Endogenous Steroids from Serum Prior to UHPLC-MS/MS Analysis

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Background: This work details sample preparation options for a panel of endogenous steroids from serum by LC-MS/MS. MRM transitions, chromatography and mobile phase additives were studied in positive and negative ionization modes. Emphasis was placed on the sample preparation to provide highly reproducible recoveries while minimizing matrix effects. Solid phase extraction was compared to supported liquid extraction in terms of recoveries, ion suppression, phospholipid content, calibration curve performance and overall sensitivity. **Methods:**LC-MS/MS analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple-quadrupole MS. MRM transitions were selected using the most intense precursor ions in positive and negative ionization using fast polarity switching. LC conditions were selected based on analyte retention, resolution, symmetry and MS signal to noise. Target analytes were spiked into human serum. Sample preparation strategies compared polymer-based reverse phase SPE, mixed-mode cation exchange SPE and supported liquid extraction (SLE). **Results:** Separation was achieved with a Restek Raptor Biphenyl HPLC column with a combination of ammonium fluoride in water and methanol. This provided better signal to noise ratios compared to acidic mobile additives in both ionization modes. Fast polarity switching was utilized with the 8060 mass spectrometer due to the inability to fully resolve analytes requiring opposite ionization modes. Investigation of non-specific binding effects to plastic collection plates during evaporation demonstrated minimal binding when using reconstitution solvents comprising high organic content, Sample preparation was optimized for the extraction of a range of endogenous steroids using polymer-based reversed phase and mixed-mode anion exchange SPE chemistries and supported liquid extraction (SLE). For each technique extraction methodology was optimized for the panel in the presence or absence of DHEAS. Inclusion of a more polar metabolite in a largely non-polar target analyte panel can present challenges when looking for optimum extract cleanliness. Recoveries greater than 75% were typically returned for each extraction protocol. Supported liquid extraction allowed matrix spiked with ISTD without any pre-treatment to be loading onto the sorbent, thus maximizing extraction volumes. Good analyte recoveries were returned when using various water immiscible elution solvents: DCM, MTBE, EtOAc and hexane mixtures. Final extraction was performed using 25/75 hexane/EtOAc when DHEAS was absent from the panel or 100% EtOAc when present. SPE optimization resulted in wash solvent compositions up to 40% MeOH depending on mechanism. Final elution was performed using EtOAc when DHEAS was absent from the panel or MeOH when present. Evaluation of phospholipid interference demonstrated SLE to remove the highest amount for both elution solvents. When using MeOH as an elution solvent in SPE, levels were far higher than when using a water immiscible extraction solvent such as EtOAc. Full results will be presented in the poster. **Conclusion:** This paper demonstrates a sensitive, fast polarity switching method for the analysis of multiple steroids from human serum. The optimized sample preparation protocols provide sufficient extraction recovery and extract cleanliness in order to reach low limits of quantitation. The development of multiple sample preparation strategies allows for a choice of workflow dependent on laboratory requirements.

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Assessment of bone health status and risk factors for fracture in type 2 diabetics

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BACKGROUND: Diabetes mellitus has profound effects on bone health. It is associated with increased glycation of bone collagen matrix that can lead to impaired bone turnover rate, decreased bone strength, increased fragility and risk of fracture. This study was conducted to determine the risk factors for fracture in T2DM patients with a view to preventing morbidity and mortality associated with bone health. **METHODS**: This case-controlled study was conducted in Lagos between July 2016 and June 2017. Participants included 90 T2DM and 77 controls made up of men and women aged 30-79 years. Physical and biochemical parameters such as BMI, WHR, phalangeal BMD, HbA1c, CTX-1, were measured in all the participants and control at first contact. Fracture risk was assessed using phalangeal BMD and FRAX. Body mass index (BMI), WHR, CTX-1 and HbA1c were measured in participants again after six months. Comparison between T2DM and controls were done using t-test and Mann-Whitney U test. Association between BMD, FRAX, anthropometric, biochemical and clinical fracture risks were done using Spearman's correlation and Chi-square tests. The level of significance was put at 5%. **RESULTS:** Phalangeal BMD did not show significant difference between T2DM and controls (p=0.230). There was no significant difference in CTX-1 levels at first contact (p=0.117), but CTX-1 was significantly lower in T2DM than controls after six months (p=0.004). The relative risks for both hip and major osteoporotic fracture are similar in T2DM and controls. (p = 0.086 and 0.243 respectively). Also, T2DM has a higher but insignificant median FRAX 10-year predictive score for developing hip and major osteoporotic fracture than the controls (p<0.757). However, the frequency of hip and major osteoporotic fractures are higher in T2DM patients than the controls. Age and duration of diabetes strongly correlate with FRAX score. (r=0.499, p<0.001 and r=0.306, p<0.001 respectively). Other clinical risk factors such as smoking, alcohol intake, cognitive and visual impairment, diabetic medications, HbA1c levels and frequent falls did not show association with fracture risk. **CONCLUSION:** The levels of CTX-1 are impaired in T2DM but with undefined phalangeal BMD. The elevated FRAX score suggests a higher fracture risk in T2DM. Subsequently, bone assessment using the above tools should be included in the routine evaluation of T2DM to determine fracture risks and complications. Appropriate intervention for high risk patients will significantly reduce morbidity and mortality in them.

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Metabolic and Biochemical Parameters in Patients with Skin Tags

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Background
Acrochordon or fibroepithelial polyp, commonly known as Skin tags (STs) are one of the most common benign skin condition, consisting of skin projecting from the surrounding skin, usually occurring on the eyelids, neck and axillae, less often on the trunk and groin. Skin rubbing, skin aging and a familial predisposition are causes for STs, while others described hormonal imbalances and hyper-insulinemia as contributing factors. Studies have found an association of STs with conditions such as obesity, diabetes mellitus and atherogenic lipid profile. Abdominal obesity and the consequent insulin resistance are said be important contributing factors for diabetes, dyslipidemia and cardiovascular disease.
Objective
To highlight the association of metabolic parameters (body mass index, blood pressure, waist circumference) and the biochemical parameters (lipid profile, fasting glucose, HbA1c and serum leptin) levels in Nepalese patients with STs visiting the Dermatology out patient department of Teaching Hospital, Kathmandu, Nepal.
Methods
This study comprised of 99(men or women) presenting to the dermatology clinic where 15 males and 35 females with STs taken as cases and 14 males and 35 females of the same age and sex with no STs were taken as controls. Metabolic parameters (body mass index, blood pressure, waist circumference) along with the Biochemical parameters (serum lipid profile, glucose, HbA1c, and serum leptin) were measured in all individuals. SPSS ver. 20.0 was used to analyze the data. Mann-Whitney U test was applied for comparison of median to see the difference between case and control group and Spearman's correlation was used to establish the association between two quantitative variables.
Results
Serum leptin was found to be significantly higher in both male and female patients having STs than the controls at the probability level of 0.001. Also, serum leptin is seen to increase with increasing BMI in both male and female cases and controls. In male with STs fasting blood glucose, glycosylated hemoglobin, triglyceride, systolic blood pressure and diastolic blood pressure was found higher than the individuals without STs. In female fasting blood glucose, glycosylated hemoglobin, total cholesterol, triglyceride, systolic blood pressure and diastolic blood pressure was found higher than the individuals without STs.
Conclusion
In the present study, there is significant association of STs with triglycerides, total cholesterol, blood pressure and serum Leptin levels. It is thus implied that skin tags may be one of the important skin markers of metabolic disorders and may attract physicians and dermatologist for further investigation as it is proved to be not just a cosmetic problem. This leads us to recommend the change of life style of patients with STs and or hyperlipidemia, as stopping active smoking and prevention of passive smoking, regular exercises, weight reduction, changing carbohydrate diets into high protein diets. Knowing that diets rich in polyunsaturated fatty acids as olive oil, omega 3, 6 and 9 fatty acids supplementation can decrease the risk of coronary atherosclerosis, we recommend their use for patients with STs and/or hyperlipidemia.

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Vitamin D status in Bangladeshi population

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Background: Vitamin D deficiency is a global public-health concern. Poor Vit-D status has been observed in South Asian populations. The cultural practices, lack of scopes and food habit do not facilitate the adequate sun exposure. Deficiency of Vitamin D indicated by low serum concentration of 25 hydroxy vitamin D [25(OH) D]. The synthesis of Vit-D is stimulated by the exposure to sun light. However no information is available on Vitamin D status for the adult populations who are working as corporate officials in Bangladesh. **Methods:** A total 226 subjects were included. Vitamin D Total (25(OH)D) was assessed in a study with corporate officials of a multinational company in Bangladesh, Dhaka on October 22, 2015. In addition 120 patient samples of aged 15 to 92 yrs. from different sources and occupations were run in different time from July to October, 2015. vitamin D was assayed by Chemiluminescense Immunoassay (CLIA) using Advia Centaur XP analyzer. The Deficiency is defined by 25(OH)D less than 20 ng/mL, insufficiency by 25(OH)D 20 to 29.99 ng/mL, sufficiency by 25(OH)D 30 to 100 ng/mL and toxicity by 25(OH)D above 100 ng/mL. **Results:** Among 106 adult officials (both male and female) aged 19-58 yrs. the mean 25(OH)D of 95 was 10.58 ng/mL and other 11 was less than 4.2 ng/mL. Among the rest 120 non-corporate subjects, the mean of 25(OH)D was 14.27 ng/mL and 19 were less than 4.20 ng/mL and 2 were above 100 ng/mL. It has been observed from the study that the proportion of the total officials of 25(OH)D deficiency was 97.17% and insufficiency was 2.83%. There was not a single person of sufficient level. In the other hand 80.83% deficiency was found from non-corporate group with 11.67% insufficiency, 5.83% sufficiency and 1.67% of toxicity. **Conclusion:** Vitamin D status of Bangladeshi population was poorer than might be expected based on cultural and geographic considerations. Corporate workers are more at risk than common people. Large scale awareness program needs to be initiated to combat this major public health concern.

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Relationship Between Hyperglycemia, Inflammation And Oxidative Stress In Type-2 Diabetic Nephropathy Subjects.

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Background: Oxidative stress increased in diabetes generates ROS producing inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL-6) in renal cells, which are the factors responsible for diabetic nephropathy. The study aimed to assess the correlation of hyperglycemia in relation to antioxidant status and inflammatory markers in type-2 diabetic patients with diabetic nephropathy and compare them with diabetics without nephropathy. **Methods:** Serum levels of inflammatory markers (Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), antioxidants [glutathione reductase (GR) and glutathione peroxidase (GPx)], plasma malondialdehyde (MDA) along with other routine biochemical parameters , fasting blood sugar (FBS), urea and creatinine levels were estimated in healthy controls (n=50), diabetic subjects without diabetic nephropathy (n=50, group 1) and with nephropathy (n=50, group 2). Comparison between the groups was done using one way ANOVA and $P<0.05$ was considered to be statistically significant and correlation was done by using SPSS version 17. **Results:** Group 2 subjects have significant increase of fasting blood sugar, serum urea , creatinine, malondialdehyde and inflammatory markers (IL-6 and Tnf-α) levels with decreasing in antioxidant GPx as compared to both group 1 and healthy controls. All the parameters are showing highly significant at $P<0.05$. Fasting blood sugar was positively correlated with MDA, serum IL-6 and Tnf-α concentrations (for group 1: $r = 0.43$, $P<0.05$; $r =0.867$, $P<0.001$; $r =0.867$, $P<0.001$ and for group 2: $r = 0.47$, $P<0.05$; $r = 0.94$, $P<0.001$; $r=0.91$, $P<0.001$; respectively). Persistent hyperglycemia levels was negatively correlated with antioxidant status i.e. GPx levels (for group 1: $r = -0.68$, $P<0.001$ and for group 2: $r = -0.74$, $P<0.001$ respectively). Serum creatinine levels were positively correlated with serum IL-6 and Tnf-α only in group 2 subjects ($r = 0.75$, $P<0.001$; $r = 0.71$, $P<0.001$; respectively). Furthermore the decreasing levels of GPx were positively correlated with serum IL-6 and Tnf-α (for group 1: $r = 0.62$, $P<0.05$; $r = 0.47$, $P<0.05$ and for group 2: $r = 0.71$, $P<0.001$; $r = 0.66$, $P<0.001$ respectively) **Conclusion:** The increased levels of inflammatory markers and increased oxidative stress as evidenced by decreased antioxidant enzymes and increased cellular peroxidation products (MDA) are associated with development of diabetic nephropathy in type 2 DM. These markers could be used to predict renal progression in long standing type 2 DM.

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A Novel Method for Free 25 Hydroxy (25OHD) Vitamin D Measurement by LC-MS/MS: Free 25OHD Associated with PTH and Calcium Better than Total 25OHD

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Background: Serum 25-hydroxy vitamin D (25OHD) is widely used as a biochemical marker of vitamin D sufficiency. In circulation, 25OHD is highly lipophilic and tightly bound to vitamin D binding protein (VDBP); a smaller fraction is weakly bound to albumin and <0.1% is circulating in free form. It has been demonstrated that the majority of cells in the human body respond to the free, rather than protein-bound, form of 25OHD. Therefore, measurement of free 25OHD (F25OHD) is likely more relevant than total 25OHD (T25OHD) for assessing physiologically active vitamin D concentrations. **Methods:** We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for measurement of free 25OHD. Sample preparation was performed as follows: F25OHD (F25OHD2 and F25OHD3) were separated from the protein bound fraction using size exclusion based technique, stable isotope labeled in-

ternal standards were added, F25OHD2 and F25OHD3 were derivatized and analyzed by LC-MS/MS. The method was compared to a commercial F25OHD ELISA (Future Diagnostics, The Netherlands). Concentrations of calcium (Ca) and parathyroid hormone (PTH) were determined using Cobas 8000 analyzer (Roche Diagnostics); T25OHD (T25OHD2 and T25OHD3) was measured using LC-MS/MS. As part of the method evaluation we analyzed F25OHD and T25OHD in a set of samples from self-reported healthy adults (n=251, 122 men and 129 women; age range 20-63; PTH range 14-112 pg/mL; Ca range 8.4-10.6 mg/dL), and set of residual patient serum (RS) samples representing a wide range of PTH and Ca concentrations (n=160, 65 men and 95 women; age range 18-85; PTH range 10-2244 pg/mL; Ca range 5.2-14.6 mg/dL). **Results:** The lower limit of quantification for F25OHD2 and F25OHD3 was 0.005 ng/mL; total imprecision at concentrations >0.01 ng/mL was <15%. Reasonably good correlation (r^2=0.787, n=62) was observed with the F25OHD ELISA, however, concentrations averaged 6.2 times lower than by the LC-MS/MS method. One likely explanation for the lower concentrations is irreversible binding of F25OHD to the labware used in the ELISA. In samples from healthy adults, we observed a better association between PTH and F25OHD (p=0.0022) than with T25OHD (p=0.082). In the RS, we observed a statistically significant association of PTH with F25OHD (p=0.015) and T25OHD (p=0.0011). In the RS group, statistically significantly lower F25OHD and T25OHD concentrations were observed in samples with Ca concentrations below 8.4 mg/dL as compared to samples within the Ca reference interval (8.4-10.2 mg/dL). Lower concentrations of F25OHD were also observed in samples with Ca concentrations above 10.2 ng/mL (p=0.05), compared to samples with Ca concentrations within the reference interval, while no association with T25OHD was observed in this group. It is known that in individuals with hyperparathyroidism and hypercalcemia, low concentrations of 25OHD could have a protective effect to prevent further increases in Ca. **Conclusion:** Our data suggest that the presented method is specific for measurement of F25OHD. Sensitivity is sufficient for quantitative measurements of F25OHD in serum samples at concentrations expected in both health and disease. Importantly, better association was observed between Ca/PTH and F25OHD than with T25OHD, indicating that F25OHD measurements likely reflect the most physiologically relevant form of vitamin D.

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Risk Index of Gestational Diabetes as Screening Tool to Avoid Glucose Challenge Test

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Background: The two steps diagnostic strategies for gestational diabetes mellitus are based on one hour glucose challenge test (GCT), used as screening tool, followed by a glucose tolerance test (GTT) when positive. The sensitivity of GCT as screening tool can be similar to other biomarkers, as fasting glucose (FG), and its reproducibility is low, especially when compared to other related biomarkers such as glycosylated hemoglobin (HbA_{1c}). Also pregnant women must stay at the consultation room one hour to complete the test and suffer discomfort and nausea.The aim of this study is to establish a risk index of GDM (RI_{GDM}) based on three biomarkers: $HbA_{1c,}$ FG and triglycerides (TG), to been used as a screening test to avoid the used of GCT. **Methods:** This was a prospective study with 507 pregnant women between the 24th and 28th weeks of gestation. All the population was submitted to GCT, and 100 g, 3 hours GTT to those with GCT ≥ 140 mg/dl. Also we determined Hba_{1c} (G8® from Tosoh), FG and TG (Cobas ® 8000 from ROCHE). GDM was diagnosed following the National Diabetes Data Group criteria. A multivariate logistic regression was used to obtain the parameters of the model equation. ROC curve was plotted, area under the curve was calculated (AUC) and cut-off points were established to optimized sensitivity(S) and specificity(SP). For each cut-off we determined S, E, positive and negative predictive values (PPV, NPV), positive and negative likelihood ratios (+LR, -LR), and number and percentage of pregnant women that wouldn't need GCT. SPSS 20 was used. **Results:**

$$RI_{GDM} = 1000x[1/1+e^{(18.9-0.078FG-1.62HbA_{1c}-0.009TG)}]$$

Utility	Low Risk		High Risk	
AUC (95% CI)	0.912 (0.847-0.978)			
Cut-off	≤20.3	≤8.5	≥710	≥112
S (95% CI) (%)	95.8(79.8-99.3)	100(86-100)	58.3(38.8-75.5)	12.5(4.3-31)
SP (95% CI) (%)	59(54.5-63.3)	26.4(22.6-30.5)	95.2(92.9-96.8)	99.4(98.2-99.8)
PPV (95% CI) (%)	10.5(7.1-15.3)	6.38(4.3-9.3)	37.8(24.1-53.9)	50(18.8-81.2)
NPV (95% CI) (%)	99.65(98-99.9)	100(97-100)	97.8(96.1-98.8)	95.8(93.6-97.2)
+LR (95% CI)	2.34(2.04-2.68)	1.359(1.29-1.43)	12.15(7.19-20.45)	20.83(4.24-93.5)
-LR (95% CI)	0.071(0.01-0.48)	0	0.44(0.27-0.70)	0.88(0.75-1.02)
No GCT, N(%)	283 (56.37)	126 (25.1)	37(7.37)	6 (1.2)
	False Negatives		Negative GCT	
N	1	0	13	2

Conclusion:
By using RI_{GDM} as screening prior to GCT, pregnant women can be classified at low risk of GDM, avoiding to perform up to 25% of GCT, with a S= 100%, or up to 56%, with a S= of 95.8%.

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The correlation Regression Model between HbA1c and Glycated Albumin in Chinese population

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Abstract:
[**Objective**]The measurement of HbA1c(Glycosylated hemoglobin) by HPLC is affected by the amount and the quality of red blood cells and some hemoglobin diseases. It is reported that the Glycated albumin(GA) is better than HbA1c in reflecting short term mean glycemia. This research focus on the correlation of GA and HbA1c and establish the fomula to estimate HbA1c by measuring the GA value. [**Methods**]20,381 subjects were recruited, including 10215 males (47 ± 12 years old) and 10,166 females (42 ± 12 years old). Using residual analysis to delete the outliers of HbA1c and GA. when HbA1c ranged from 4.0% to 12.0%, corresponding to GA ranged from 7.5% to 45%. HbA1c values between 4.0% to 8% were divided into 8 groups: <4.5%, 4.5% -5%, 5% -5.5%,5.5% -6%, 6% -6.5%,6.5% -7%, 7% -7.5%, 7.5% -8%, respectively compared the difference of GA values corresponding to HbA1c. HbA1c values during 4.0% to 8.0%were divided into 38 groups as HbA1c increases by 0.1%. The scatter plot of GA average as X-axis and HbA1c as Y-axis, Calculate the correlation between HbA1c and GA and analysis the ratio of GA / HbA1c in each group. [**Results**]The levels of GA and HbA1c have no significant difference between male and female was shown in table.1). All the data were analyzed as scatter plots, and the equation that reflects the correlation between the GA and HbA1c was HbA1c=0.181GA + 3.489,R^2 = 0.299 (Figure 1). It was found that the linear relationship between GA average and HbA1c of increases by 0.1% was discontinuous at HbA1c = 6.2%(GA = 12.28%), which is a turning point(Figure 2), using this breakpoint as a boundary and do the piecewise equation (Figure 3).Before and after the breakpoint equation is : when HbA1c <6.2% or GA <12.28%, the formula is: HbA1c = 1.136GA-7.289, R2 = 0.824; when HbA1c≥6.2% or GA≥12.28%, the formula is: HbA1c = 0.252GA + 3.163, R2 = 0.948. [**Conclusion**] There is a discontinuous linear relationship between HbA1c and GA. When HbA1c is 6.2%, there is a significant turning point between GA and HbA1c. Any factors that affect the amount and quality of hemoglobin will interfere with the results of HbA1c, when HbA1c and blood glucose monitoring results can be inconsistent with the evaluation of HbA1c by measuring The GA value.

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Analytical Determination of Testosterone in Human Serum using an Ultivo LC/TQ

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Background: In this research study, a robust, sensitive and relatively fast analytical method was developed for the quantitation of free testosterone in serum using an Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ). Ultivo was designed to address many challenges faced by research laboratories and this research study was conducted in order to assess how this novel triple quadrupole mass spectrometer (MS) could perform with a typical endogenous analyte of research interest. Innovative technologies within Ultivo allowed for a reduction in overall physical footprint, while generating comparable analytical performance to similar, but physically larger MS systems. Innovations such as, VacShield, Cyclone Ion Guide, Vortex Collision Cell, Virtual Pre/Post Filters and small Hyperbolic Quads supported a reduction in instrument size, yet maximized quantitative performance, instrument reliability and robustness. Furthermore, Ultivo reduces the overall frequency for system maintenance, making the system operation and maintenance manageable for non-expert MS users. MassHunter Software simplifies data acquisition, method set up, data analysis and reporting, which results in the fastest possible acquisition-to-reporting time, increasing lab productivity. Herein, this research study aims to outline typical confirmation performance of free testosterone in human serum using the Ultivo LC/TQ. Lower limits of quantitation, chromatographic precision and calibration linearity, range and accuracy will be discussed. **Methods:** Sample analysis was performed using an Agilent 1290 UHPLC/Ultivo LC/TQ with electrospray ionization (ESI) in positive mode. The chromatographic column used was a Poroshell EC C18 column (2.1 x 50 mm w/ 2.7 µm). The UHPLC mobile phases were 0.1% formic acid and 5mM ammonium acetate in water (A) and methanol (B). The total chromatography cycle time was 6 minutes. Two MRM transitions are monitored for the analyte and a single transition for the deuterated internal standard. Human serum samples (250 µL) were spiked with calibrators at various concentration levels, cold acetonitrile (500 µL) containing the deuterated internal standard was added to affect protein precipitation and centrifuged at 5000 rpm. The supernatant liquid was then further diluted (1:2) with a 50:50 methanol:water solvent mixture prior to instrument injection. **Results:** Excellent linearity and reproducibility were obtained, with a concentration range from 1.0 pg/mL to 100 ng/mL (20 fg to 2000 pg on-column) for the testosterone with a linearity coefficient of > 0.999 for three batches prepared for this research study. Precision data observed over the three batches resulted with a %RSD variation of < 12% across all calibration levels in this research study. **Conclusion:** This research project demonstrates that the performance of the Ultivo LC/TQ with the analytical methodology described herein generated excellent linearity, precision and sensitivity across the range of 1.0 pg/mL through 100 ng/mL for free testosterone in human serum within an analysis cycle time of 6 minutes. Further research is needed before implementing in a routine clinical setting. For Research Use Only. Not for use in diagnostic procedures.

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Anti-thyroid peroxidase and anti-thyroglobulin antibodies positivity in patients with hypothyroidism - is it necessary to ask for both antibodies in the evaluation of autoimmune thyroid disease?

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Introduction: Thyroid is a common target for autoimmune diseases (AID). Thyroid peroxidase antibodies (TPOAb) have been involved in the tissue destructive processes associated with the hypothyroidism observed in Hashimoto's and atrophic thyroiditis. There is some debate over the clinical utility of serum thyroglobulin antibodies (TgAb) measurements, since they do not appear to be a useful diagnostic test for AITD In areas of iodide sufficiency. The isolated positivity of TgAb showed no association with hypothyroidism or TSH elevations. **Objective:** To evaluate the prevalence of TPO and Tg antibodies positivity in patients with TSH levels higher than 10 mIU/L. **Methods:** We analyzed samples of both genders ≥ 12 years from a large database of a private reference clinical laboratory, tested for TSH, TPOAb and TgAb (ECLIA, Modular, Roche) in the period from January to December 2016. All the patients had TSH levels higher than 10 mIU/L. TPOAb and TgAb values, respectively, above 34 U/mL and 115 U/mL were considered positive. **Results:** 771 patients were evaluated, 72% women, mean age 52 ± 20 years; 316 (41%) of the patients had both negative antibodies; 455 (59%) presented positive TPOAb and/or TgAb. Analyzing these 455 patients, we found that 262 (58%) showed positivity of both antibodies; 147 (32%) only positive TPOAb and 46 (10%) only positive TgAb. 409 (90%) presented positive TPOAb regardless of TgAb levels (positive or negative) and 308 (68%) presented positive TgAb regardless of TPOAb levels. **Conclusions:** We found a higher positivity of TPOAb comparing with TgAb in patients with hypothyroidism, TSH higher than 10 mIU/L. Most of the patients that were TgAb positive were also TPOAb positive. Measurement of TPOAb only, allowed the diagnosis of thyroid autoimmunity in 90% of patients, suggesting that concomitant measurement of TPOAb and TgAb may be dispensable in routine evaluation of thyroid autoimmunity.

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Commutability of processed materials with different matrix for progesterone measurement

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Background: the measurement of progesterone is important to determine ovarian function and to predict early pregnancy. The results from common External Quality Assessment (EQA) program differ greatly. To better interpret the EQA results and investigate the possibility of preparing commutable materials for EQA program or preparing candidate reference materials, the present study evaluated the commutability of reference materials, EQA materials, swine sera, human serum pools prepared from patient samples and hydroxypropyl-beta-cyclodextrin aqueous solution. **Methods:** an ID/LC-MS/MS method for progesterone measurement was used as comparative method. Six immunoassays (Abbott, Beckman, Chivd, Mindray, Roche, Siemens) that were commonly used in clinical laboratories were chosen as evaluated methods. Thirty-five processed materials were tested along with forty-eight individual patient serum samples. All of the samples were measured in triplicate for the routine immunoassays. The samples were tested in order then reversed. A scatter plot was generated from patient samples, and 95% prediction intervals were calculated to evaluate the statistics commutability of the processed materials. **Results:** Ordinary linear regression (OLR) was performed and the slopes of the regression lines were 0.961~1.263 and the intercepts were -1.136~0.891. The OLR and its 95% confidence intervals demonstrated that reference materials (ERM-DA347, BCR-348R, GBW09197, GBW09198, and GBW09199) were commutable for all the six immunoassays tested. The hydroxypropyl-beta-cyclodextrin aqueous solution exhibited negative matrix effects in all immunoassays. Swine sera exhibited positive matrix effects in some immunoassays. Part of EQA materials showed positive matrix effects in some immunoassays. **Conclusion:** The reference materials and human serum pools prepared from patient samples were commutable. Non-commutability of the tested EQA materials was observed among current progesterone immunoassays, which implied that interpretation of EQA results needs consideration of the bias caused by non-commutability. Other materials such as hydroxypropyl-beta-cyclodextrin aqueous solution and swine sera were mostly non-commutable and could not be used as candidate reference materials.

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The Circadian Rhythm of Cortisol in the saliva ofpatients with mild traumatic brain injury-Comparison with healthy controls

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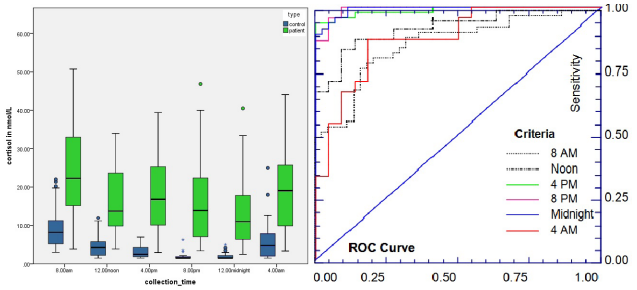
Background: Traumatic brain injury (TBI) patients represent a specific subgroup of trauma population due to activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Salivary cortisol is an accepted surrogate for serum free cortisol in the assessment of HPA-axis function. The purpose of this study was (1) to establish the feasibility of saliva cortisol measurement in mild-TBI patients, and (2) to determine the diurnal pattern of saliva cortisol in the acute phase after injury. Methods: Saliva cortisol was measured with an electroluminescent immunoassay on Cobas e-411 (Roche, Mannheim, Germany). Saliva samples were collected

and stored, according to manufacturer's specifications until tested. Saliva samples were prospectively collected from 12 mild-TBI patients (GCS=15). 11 healthy volunteers served as controls. All patients and controls were males and their mean age(±SD) was 59.4 (12.1) and 41.2 (18.9) years respectively. Collections in both patients and controls were performed on 4 consecutive days during the first week after injury, and 6 times during a day at 4AM, 8AM, Noon, 4PM, 8PM and Midnight. Results: Median saliva cortisol concentrations were significantly higher in patients versus controls at all time points ($p<0.001$) as shown at the left side of our graph. These levels remain elevated, compared to controls, during the whole follow-up period. Patients develop the expected PM versus AM decrease in cortisol concentration seen in controls ($p=0.005$). ROC-curve analysis was performed for each collection time point for patients vs. controls. Area under the curve was significantly higher ($p<0.05$) at PM versus AM collections (right side of graph). Conclusion: Our data show that in mild-TBI the HPA is activated, the diurnal pattern of saliva cortisol is maintained as seen in controls, and finally the best sampling time for saliva cortisol measurement is between 8PM and Midnight.



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Performance of selected Fertility Panel Immunoassays (FSH, LH, Progesterone, Prolactin) on the Alinity i platform, Abbott´s Next Generation Immunochemistry System

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Background: Abbott offers a range of assays in the fertility panel on the Alinity i system that can assist healthcare professionals in the diagnosis and management of fertility issues by providing reliable and accurate results. Abnormal levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) may be indicative of potential gonadal failures and/or a dysfunction of the hypothalamic-pituitary axis. Abnormal progesterone levels are indicators for reproductive disorders such as infertility or pregnancy loss. Quantitation of prolactin may be useful the diagnosis of male female gonadal and pituitary dysfunctions and in the management of amenorrhea and galactorrhea. The aim of the current study is to evaluate the precision, lower limits of measurement (Limit of Blank, LoB; Limit of Detection, LoD; Limit of Quantitation, LoQ) and method comparison as key performance characteristics of the four selected assays on the newly developed Alinity i system. **Methods:** Studies to determine the lower limits of measurement and the precision of the assays on the Alinity i system were conducted based on guidance from CLSI EP17-A2 and CLSI EP05-A2, respectively. The Alinity i assays were also tested side by side with the corresponding ARCHITECT assays to generate method comparison data based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method. **Results:** The observed results for precision, lower limits of measurement and method comparison for the fertility panel assays on Alinity i are shown in the table below.

Assay	Unit of measure	Within-Laboratory (Total) Imprecision	LoB	LoD	LoQ	Method comparison (Slope / Correlation)
FSH	mIU/mL (IU/L)	1.9 - 2.7 %CV	0.01	0.02	0.11	0.98 / 1.00
LH	mIU/mL (IU/L)	2.8 - 4.7 %CV	0.02	0.04	0.12	0.94 / 1.00
Progesterone	ng/mL (nmol/L)	3.1 - 6.1 %CV (3.0 - 5.8 %CV)	0.1 (0.3)	0.2 (0.6)	0.5 (1.6)	0.95 / 0.99
Prolactin	ng/mL (mIU/L)	2.1 - 2.8 %CV (2.1 - 2.8 %CV)	0.45 (9.45)	0.47 (9.87)	0.79 (16.59)	1.03 / 0.99

Conclusion: The selected assays demonstrated satisfactory performance in terms of precision and lower limits of measurement on the Alinity i system. Method comparison data showed very good correlation between the Alinity i assay and the respective ARCHITECT assay.

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Thyroid Auto-Antibodies - Impact of Change in Assay Methodology on Thyroid Testing

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Background: Autoimmune thyroid disease (AITD) causes cellular damage and alters thyroid gland function by humoral and cell-mediated mechanisms. AITD, commonly Graves' Disease and Hashimoto's Thyroiditis, is usually accompanied by the presence of thyroid autoantibodies such as anti-thyroglobulin antibody (TgAb), anti-thyroperoxidase antibody (TPOAb) and anti-thyroid-stimulating-hormone-receptor antibody (TRAb). Assays for thyroid autoantibodies suffer from analytical specificity and standardisation limitations, resulting in wide differences in test results and reference limits. Our study evaluated the Roche TgAb, TPOAb and TRAb immunoassays on the Roche Cobas e602 as an alternative to the Brahms Compact Plus immunoassays (TgAb, TPOAb) and the Brahms TRAK radio-receptor assay (TRAb). We also examined the reference intervals of these autoantibodies in local healthy volunteers. **Methods:** Performance validation parameters of the Roche assays included assay imprecision, lower limit of detection, linearity and carry-over. Method correlations between Cobas e602 assays and Brahms Kryptor Compact Plus (TgAb,TPOAb) / Brahms TRAK radio-receptor assay (TRAb) were tested using patient serum samples (n=130-190). Inter-assay concordance between Roche and Brahms were evaluated using the respective manufacturers' cutoffs. Reference intervals for the thyroid autoantibodies were assessed on the Roche assays in metabolically stable local healthy volunteers, using screening criteria of serum thyroid stimulating hormone (TSH) levels between 0.5-2.0 mIU/L, no personal or family history of thyroid diseases and absence of non-thyroid autoimmune diseases. **Results:** Within-run and total imprecision for the Roche thyroid autoantibody assays were determined to be ≤7.9%. All 3 assays demonstrated linearity across the manufacturer's analytical measurement range (recoveries: 96 to 125%). Lower limits of detectable concentrations of TgAb (2.2 IU/mL), TPOAb (2.3 IU/mL), TRAb (0.2 IU/L) agreed with manufacturer's claims. Results of carry-over studies were insignificant. Method correlation with the Brahms assays revealed Passing Bablok regression slopes of 2.87, 0.20, 0.87 and intercepts of -68.1, +7.1, -0.03; mean bias (Altman Bland) of +10.9%, -88.9%, -14.9%, Spearman's correlation coefficients of 0.72, 0.88, 0.90 for TgAb, TPOAb and TRAb respectively. Assay concordance for positive/negative results were TgAb (82.0%), TPOAb (92.8%) and TRAb (90.4%), with Cohen's kappa values of 0.64, 0.85 and 0.80 respectively. Non-parametric estimates of upper reference limits (97.5th) of the local volunteer subjects (n=158, M:F ratio=1:2, age range 19-65 yrs old, median age 32 yrs old) were TgAb (107 IU/mL) were TPOAb (30 IU/mL) and TRAb (0.82 IU/L) respectively; distributions were non-Gaussian, with ≥ 98 % of the population falling under manufacturer's upper reference limits of TgAb (115 IU/mL), TPOAb (34 IU/mL) and TRAb (1.75 IU/L). **Conclusion:** Overall, Roche TgAb, TPOAb and TRAb assays showed acceptable analytical performance and represent good alternatives to our current Brahms assays. Notwithstanding wide differences in absolute results between different assay methodologies, substantial concordance was observed with the use of manufacturers' method-appropriate cutoffs. We validated the manufacturer's upper reference limits and found them applicable to our local population. Laboratories looking to switch thyroid autoantibody assays should carefully evaluate the impact of change on patient testing and transferability of the manufacturer's expected values to its own patient population.

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Quantitative Proinsulin Assay by Electrochemiluminescence on the Meso Scale Diagnostics Platform

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Background: Proinsulin is the precursor of insulin and c-peptide. Proinsulin measurements, in conjunction with insulin and c-peptide, are useful in the diagnostic workup of hypoglycemia and suspected insulinomas. **Objective:** Develop an assay for proinsulin quantitation in human plasma using the electrochemiluminescent multi-array technology from Meso Scale Diagnostics (MSD, Rockville, MD). **Method:** The proinsulin assay is a sequential two-site electrochemiluminescent laboratory-developed test. A biotinylated mouse monoclonal antibody against insulin and proinsulin is

bound to a streptavidin coated 96 well plate, followed by a two hour 25 mcL sample incubation at room temperature. After a wash step, there is a one hour incubation with a SULFO-TAG™ labeled goat polyclonal antibody. After another wash step, an MSD read buffer, containing tripropylamine, reacts with the SULFO-TAG and the electrochemiluminescent signal is captured by the CCD camera of the MESO QuickPlex SQ 120(MSD QuickPlex) instrument. The signal is directly proportional to the amount of proinsulin in the sample. The assay is calibrated against the WHO 1st International Standard for Human Proinsulin (NIBSC code: 09/296). The performance characteristics of the assay were established using at least three different reagent lots. Method validation included determination of imprecision, limits of detection and quantification, analytical measurement range (AMR), accuracy by spike recovery, interferences, effect of antigen excess, and a method comparison with the current Mayo laboratory-developed assay. **Results:** Intra-assay and inter-assay imprecision on human EDTA plasma pools (1.4-27 pmol/L) ranged from 2.0-3.4% and 6.6-10.3%, respectively. The assay limit of detection was 0.03 pmol/L, using calculations suggested by International Union of Pure and Applied Chemistry Compendium of Analytical Nomenclature. A precision profile (~0.1-2.6 pmol/L) established a limit of quantitation of 0.6 pmol/L (%CV=15.3). The AMR was 0.6-350 pmol/L (slope of 0.99, intercept of -0.3, and R^2 of 0.98). Spike recovery using WHO 1st International Standard for Human Proinsulin in human EDTA plasma was 93% (range 88-97%). The assay was not affected by concentrations of hemoglobin ≤1228 mg/dL, triglycerides ≤942 mg/dL, or bilirubin ≤57mg/dL. Antigen excess did not affect the assay up to 3234 pmol/L. Competition studies with insulin showed that an insulin concentration up to 3472 pmol/L would not affect proinsulin recovery. Insulin, c-peptide, and Lispro do not cross-react in the assay. Stability studies on freshly collected EDTA plasma showed proinsulin is stable for 8 hours ambient, 3 days refrigerate, and 90 days frozen. Comparison with the in-house proinsulin assay (n=94, range: 1.2-350 pmol/L) showed an R^2 of 0.98, slope of 0.99 and intercept of 0.22 by Passing-Bablok regression fit. The reference interval for proinsulin was established by testing 94 healthy individuals (29 males and 65 females) and calculated using quantile regression to be 3.6-22 pmol/L. **Conclusion:** We have developed a proinsulin electrochemiluminescent assay on the MSD QuickPlex standardized against the WHO 1st International Standard for Human Proinsulin. The following advantages are observed when compared to the current in-house assay: broader analytical measurement range (0.6-70 pmol/L to 0.6-350 pmol/L); 10-fold decrease in sample volume (250 mcL to 25 mcL); and shorter turn-around time (1.5 days to 6 hours).

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Validation of intact hCG AutoDELFIA assay for use in urine samples

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Background: This analysis sought to examine the analytical performance characteristics of an intact human chorionic gonadotrophin (hCG) assay (Perkin Elmer) for use on urine samples on the AutoDELFIA analyser. It was desired that performance would be suitable to enable analysis of hCG concentration in biobanks of urine samples from early pregnancy and to assign concentration to hCG standards used for validation of home pregnancy tests. **Methods:** Limit of blank and Limit of detection were determined following the principles outlined by Pierson-Perry, using 2 reagent lots, 1 instrument system (AutoDELFIA 1235 autoanalyser), 3 test days, a pooled blank sample, 5 low level samples and at least 7 replicate measurements per sample for each reagent-day combination. The pooled blank sample was created by pooling urine from non-pregnant women and passing through an anti-hCG immunoadsorption column to remove all hCG. Samples were prepared from dilution of WHO 5th International Standard for intact hCG. Instrument signals were converted to analyte values through offline calibration to avoid censoring of data for blank samples. Limit of quantitation was calculated via a total error approach, and used 8 low level samples. High dose hook, assay drift, cross-reactivity and interfering substances profile were also examined, as was effect of sample dilution. **Results:** Limit of blank for hCG assay was 0mIU/ml for each reagent lot. Low level sample results (0.25, 0.5, 1, 2, 3mIU/ml, n=28/sample) were used to determine limit of detection as being 0.17mIU/ml. A minimum of 60 repetitions/sample were required to determine limit of quantitation. Total Error was >36% at very low hCG concentrations (0.25, 0.5, 1mIU/ml), whereas total error was <17% for hCG concentrations of 2, 3, 5, 10, 25mIU/ml; so limit of quantitation was deemed to be 2mIU/ml. The linear range of the assay was 0-5000mIU, with samples of higher concentration requiring dilution in order to return accurate results. Dilution of samples retuned results that were 104%±3.13 from expected concentration. No high dose hook or assay drift was observed, nor was there cross-reactivity to species with high homology (LH, FSH, TSH). **Conclusion:** The intact hCG AutoDELFIA assay was found to have suitable analytical performance for use in urine samples and for assigning concentration to reference standards.

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Interference in 25OH Vitamin D Assay in a patient with Multiple Myeloma Disease

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Background: : Currently automated immunoassays are the most commonly techniques to measure Vitamin D (25-OH VitD). Different studies reported possible interferences in Multiple Myeloma Disease due to immunoglobulins present in the patient' s serum, wich could lead to a wrong laboratory result and also an erroneous diagnosis. The possibility of methodological interference must be taken into account when: the laboratory result shows lack of coherence with the clinical presentation, the presence of an unusual analyte concentration and also discordant results measured by different analytical methods. **Methods:** We reported a case of a 74 years old male with Myeloma disease, he presented high levels of 25-OH VitD concentration without any clinical sign of toxic levels. He denied any oral Vitamin D supplements or any other multivitamin preparation and neither sun exposure. The following analytes were: PTH and 25OH VitD (Reference Values (RV): Sufficiency more than 30 ng/mL, Deficiency minor than 10 ng/mL, insufficiency 10-30 ng/mL, toxicity more than 100.0 ng/mL) were processed by Architect i2000 (CLIA by Abbott), Gamma Globulins (Nephelometry by Beckman), Calcium and Phosphorus (Colorimetric and *Ammonium phosphomolybdate* respectively by Beckman), Alkaline phosphatase (UV kinetic by Beckman), and Reumathoid Factor (Aglutination).To confirm the presence of interferences the serum was diluted to check linearity and then treated with polyethylene glycol (PEG) (25%), to separate by precipitation high molecular weight forms. **Results:** The patient showed 25-OH VitD concentrations greater than 150.0 ng/mL. Non-linearity dilutions (Dil) (Dil 1:2= 17.8 ng/ml, Dil 1:5= 17.4 ng/mL) were found, wich suggest the presence of interferences. The 25OH VitD concentration post treatment with PEG was 17.6 ng/ml, wich confirm the presence of immune complexes. Those results were confirmed in another sample of the patient (25-OH VitD:180 ng/mL and 25 OH VitD post PEG:16.4 ng/ml). On the other hand the result of IgG: 6520 mg/dL (RV: 800-1700) and the protein electrophoresis showed a monoclonal peak in Gamma 4.76 g/dL, confirm the high concentration of immunoglobulins. **Conclusion:** Assay interference should be considered in unexpected abnormal results of 25OH Vit D levels in presence of Myeloma disease. Laboratory´s staff should contemplate the use of additional tools to detect and eliminate these kind of artifacts. Communication with physicians is very important for patient follow-up.

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An evaluation of hemoglobin A1C measurement by dried blood spots

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Background: Diabetes is a chronic metabolic disorder of sustained high blood sugar levels that carries a high cost in resources, quality of life and mortality. Due to changing lifestyles and diets, its global prevalence is growing especially in low- and middle-income countries. Glycated hemoglobin (HbA_{1C}) levels represent a patient's mean serum glucose level over the previous 3-4 months and is the preferred test for diabetes diagnosis and monitoring. Clinical testing by dried blood spot (DBS) is an approach that has numerous advantages over conventional phlebotomy including a small volume requirement, simple collection that can be performed by the patient at home, nonhazardous transportation and handling, compact storage, and long stability in the absence of refrigeration. The measurement of HbA_{1C} from DBS is therefore attractive for widespread diabetes testing and monitoring, especially in locales with access challenges to testing facilities. Here, we assessed DBS as the specimen of choice for HbA_{1C} measurement by employing a simple extraction method in combination with the Roche Cobas c 513 analyzer (Roche Diagnostics). **Methods:** 65 µL EDTA whole blood samples rewarmed to room temperature were spotted on Whatman® 903 Protein Saver cards (Sigma-Aldrich) and air dried for 4-12 hours. Samples were then extracted by 6.00 mm diameter hole punches and resuspension in 1 mL of Hemolyzing Reagent for Tina-quant® HbA_{1C} Gen. 3 immunoassay (Roche Diagnostics) which contains tetradecyltrimethylammonium bromide detergent. After 30 min at room temperature with periodic gentle inversions, samples were spun for 1 min at 14,000 rpm in a microcentrifuge for final sample extraction. The recovered supernatants were aliquoted to false bottom tubes for testing on the Roche Cobas c 513 analyzer via the hemolysate application. Within run precision of this DBS method was evaluated using 20 repetitions of a normal sample (5.8%HbA_{1C}) and a pathological sample (10.5%HbA_{1C}). Method accuracy was determined by spotting and extracting

stored proficiency samples from the College of American Pathologists. For direct comparison with routine whole blood testing, 40 samples representing a range of 4.6% to 14.0%HbA_{1C} as measured by the former method were spotted, extracted and analyzed. **Results:** Using the DBS extraction procedure, within run precision of HbA_{1C} measurement was 0.4% and 0.8%CV for normal and pathological leftover patient samples, respectively. Measurement of proficiency survey samples by DBS extraction found a low average bias of 0.01% ($y = 1.03x - 0.20$; $R^2 = 0.999$). Strong correlation was also observed in comparison runs between the DBS method and routine phlebotomy-based whole blood HbA_{1C} testing using the same instrument ($y = 0.98x + 0.21$; $R^2 = 0.999$). In these runs, samples showing relatively high bias were scattered across the measuring range and showed no clear systematic pattern. Furthermore, the associated biases remained within the total allowable analytical error (2.0%) with absolute biases no greater than 0.4%HbA_{1C}. **Conclusion:** The simple DBS extraction method partnered with the Roche Cobas c 513 is reproducible, accurate and robust for HbA_{1C} analysis. This supports the implementation of this convenient specimen collection approach for wide-reaching diabetes testing at a population level.

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Development of prototype renin concentration assay which well-correlate with renin activity under the treatment with direct renin inhibitor

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Background: Renin controls blood pressure via renin-angiotensin-aldosterone system. Measuring renin concentration is useful to screen primary aldosteronism from large number of hypertensive patients. There are two forms of assays to measure renin in plasma currently used in clinical settings, plasma renin activity (PRA) and plasma renin concentration (PRC). Although PRA assays offer better sensitivity, they also have disadvantages such as the dependence on the plasma concentration of renin substrate angiotensinogen and the difficulties in sample management. On the other hand, PRC assays have advantages in terms of rapidity and easy sample management and several active renin specific PRC assays have been commercially available. However, these PRC assays are known to have poor correlation with PRA under treatment with direct renin inhibitor, aliskiren, because renin specific antibody used in the current commercial PRC assays detects aliskiren bound renin and prorenin as active renin, despite their inactivation by aliskiren binding. In this study, we aimed to establish PRC assay which highly correlates with PRA even for the patients under the treatment with direct renin inhibitor. Methods: Anti-renin antibodies were established by mice immunization and renin specific antibodies were selected based on specificity to renin. These antibodies were characterized for the reactivity to renin in the presence or absence of aliskiren. Renin specific sandwich ELISA with these antibodies was evaluated by reactivity to renin and prorenin and correlation with renin activity. Result: Several monoclonal antibodies which showed high specificity to renin were successfully obtained. Cross reactivity to recombinant prorenin of these antibodies was less than 10% of reactivity to recombinant renin. It was found that one of the highly renin-specific antibodies lost reactivity to recombinant renin in the presence of aliskiren in the concentration dependent manner, while the reactivity of other antibodies was not affected. Moderately renin-specific antibody which showed approximately 50% of cross reactivity to prorenin was also obtained. The sandwich ELISA established using highly renin specific and aliskiren-sensitive antibody and moderately renin specific antibody showed not only high specificity to active renin, but also aliskiren sensitivity. Further analysis revealed that this aliskiren sensitive ELISA assay showed high correlation with renin activity as measured by in-house renin activity assay using fluorescent renin substrate even with aliskiren. Conclusion: We have developed an unique renin specific monoclonal antibody that loses reactivity in the presence of direct renin inhibitor, aliskiren. The measurement of recombinant renin by the sandwich ELISA developed with our aliskiren sensitive antibody showed striking correlation with those of in-house renin activity assay. Although clinical significance has yet to be revealed, our aliskiren sensitive, highly renin-specific antibody offers promising tool to develop PRC assay which reflects true plasma renin activity, and may be useful not only for the diagnosis of primary aldosteronism, but also for monitoring therapeutic effects of direct renin inhibitors.

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Frequency of insulin resistance assessed by Quantose-IR©, HOMA index and triglycerides/HDL-c ratio.

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Background. Overweight, obesity and associated co-morbidities diabetes and cardiovascular disorders have increased dramatically in Mexico. Insulin resistance (IR) is an important factor for development of diabetes. Quantose-IR© is a non-glycemic test to identify IR. **Objective.** To assess the frequency of IR by Quantose-IR©, HOMA index, and triglycerides/HDL-c (T/H) ratio, in ambulatory patients from two laboratories in Mexico City. **Methods**. A laboratory information system database search was performed to identify patients that requested a chemistry profile and Quantose-IR© (Metabolon Inc.) from January 2016 to March 2017. Patients age 18 and older were included; those with a serum glucose ≥126 mg/dL were excluded. Quantose-IR was performed at a Mexican reference laboratory and provides an IR index based on plasma concentration of α-hidroxibutiryc acid, oleic acid, 1-linoleoylglycerophosphocholine measured by UHPLC-MS-MS, plasma insulin measured by chemiluniscence and a Metabolon proprietary algorithm. Serum glucose and lipids were analyzed locally in AU5800 and Lx20 instruments (Beckman-Coulter). Cut-off values for IR were >63 for Quantose-IR, >2.5 for HOMA index and for T/H was >2.5 for women and >3.5 for men. Statistical differences were evaluated by chi2 test for categorical variables and Mann-Whitney test for quantitative variables. **Results.** Patients included were 708, 360 (51%) were females. Median age was 46 years, ranging from 18 to 85 years. The overall IR frequency was 78% for Quantose-IR, 52% for TG/HDLc ratio and 45% for HOMA index, with p-values of <0.001 and <0.0001 for Quantose vs. T/H ratio and HOMA index respectively. The frequency of IR when patients were classified according to glucose and insulin concentrations is summarized in the following table.

	N	Quantose	HOMA index	T/H ratio
Normal glucose and normal insulin	481	72%	21	54
Abnormal glucose and normal insulin	147	88%	72%	61%
Normal glucose and abnormal insulin	45	100%	100%	75%
Abnormal glucose and abnormal insulin	35	100%	100%	80%

Conclusion. Quantose-IR identifies more patients as IR notably in normal glucose and insulin patients, HOMA index is best used when insulin concentration is abnormal. T/H ratio had the lowest performance for identifying IR.

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Comparison of Five TSH-Receptor Antibody Assays in Graves' disease. Results from an observational study

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Background: Early diagnosis and relapse prediction in Graves' disease [GD] may influence treatment. We assessed the abilities of four TSH-receptor antibody tests [TRAb] and one cAMP bioassay to predict relapse of GD. **Methods:** Observational study investigating patients presenting with GD at a Swiss hospital endocrine referral center or an associated endocrine outpatient clinic. Main outcomes were diagnosis and relapse of GD after stop of anti-thyroid drugs. We used Cox regression to study associations of TRAb levels with relapse risk and calculated area under the receiver operating characteristics curve [AUC] to assess discrimination. Blood draws took place as close as possible to treatment initiation. **Results:** ROC curve analysis revealed AUCs ranging from 0.90 (TSAb Biossay) to 0.97 (IMMULITE TSI) for the diagnosis of GD. Highest sensitivity (94.0%) was observed for IMMULITE and RSR TRAb Fast while the greatest specificity (97.9%) was found with the EliA anti-TSH-R. GD relapse was studied using Cox regression analysis comparing the highest versus the lower quartiles. The highest hazard ratio [HR] was found for BRAHMS TRAK (2.98, 95% CI 1.13 - 7.84), IMMULITE TSI (2.40, 95% CI 0.91 - 6.35), EliA anti-TSH-R (2.05, 95% CI 0.82 - 5.10), RSR Fast TRAb (1.80,

95% CI 0.73 - 4.43), followed by RSR STIMULATION (1.18, 95% CI 0.46 - 2.99). Discrimination analyses showed respective AUCs of 0.68, 0.65, 0.64, 0.64, and 0.59. **Conclusion:** The assays tested had good diagnostic power and relapse risk prediction with few differences among the new assays.

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Do we need to chill samples for renin activity?

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Background:
Collecting blood on ice for renin determination reportedly results in spuriously high results due to activation of prorenin into renin which converts angiotensinogen into angiotensin 1. However when determining plasma renin activity, samples are requested to collect into pre-chilled EDTA tubes. Our objective was to assess whether samples need to be taken into pre-chilled tubes in actual condition.
Methods:
A prospective cross sectional study was performed. Patients who came for plasma renin and aldosterone assay were taken as the study sample. Peripheral venous blood was collected from 22 patients in the seated position after 2 hrs of ambulation between 08:00 and 10:00 h. From each patient blood was collected into 2 tubes containing K-EDTA (pre-chilled and room temperature EDTA tubes). Blood taken into pre-chilled EDTA tube was immediately centrifuged at 4 ^{0}C and the other tube was processed at room temperature. The interval from collection to commencement of incubation averaged between 1 to 2 hours. Radioimmunoassay of angiotensin 1 was used to determine plasma rennin activity. Angiotensin 1 was measured after 1 hr incubation at both 4^{0}C and 37^{0}C. The assay was performed in duplicates. The plasma rennin activity was calculated by the difference in angiotensin 1 found in 2 samples. For the statistical analysis, values were reported as mean ± SD (Paired t test). The difference at $P < 0.05$ was considered significant.
Results:
There was no significant difference in the mean value for plasma renin activity in samples whether processed at room temperature (3.29 ± 2.77) or 4^{0}C (3.05 ± 2.75). Samples handled at room temperature had a higher basal angiotensin 1 value (2.03 ± 4.16 Vs 1.51 ± 3.26) but, the difference was not significant.
Conclusion:
Our data indicate that use of pre-chilled EDTA tubes and refrigerate centrifuge is unnecessary to determine plasma rennin activity and at 4^{0}C, cryoactivation of prorenin does not occur rapidly.
Keywords
Renin activity, chilled EDTA tubes, prorenin

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Requirement for age-specific peak cortisol references to insulin-induced hypoglycaemia in children.

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Background: The insulin tolerance test (ITT) is frequently used for simultaneous evaluation of the hypothalamic-pituitary-adrenal axis and growth hormone secretion in children. In contrast to extensive published literature pertaining to GH response following ITT, only few reports have considered the magnitude of cortisol response to this test in pediatric population. Reference range is not clearly established for peak cortisol response to ITT despite limited data suggesting an effect of age on peak cortisol. **Objective:** To determine whether peak cortisol response to insulin hypoglycemia test in children is related to age and to try to establish pediatric reference data. **Design:** The present study was a retrospective cohort study. **Methods:** We performed a retrospective analysis of children and adolescents submitted to insulin tolerance test in a laboratory referral center over a 5-year period (2012 - 2017). Inclusion criteria were age ≤ 18 years, adequate hypoglycemia, defined as a glucose nadir ≤ 2.2 nmol/L (≤ 40 mg/dL) (4, 7) and a normal response of cortisol to the test. A normal response to the test was defined as a peak cortisol (maximum absolute concentration) at any time of the test ≥ 400 nmol/L (14.4 µg/dL). Patients with known or suspected organic hypothalamic-pituitary diseases and patients receiving glucocorticoid medication were excluded. One hundred and twenty-four subjects (86 males) met the criteria. Blood samples were collected at time 0, 30, 60, 90 and 120 min in relation to insulin bolus injection (0.075 - 0,15 U/kg). Glucose, cortisol and growth hormone were measured in all samples. **Results:** One hundred and twenty-four patients were eligible for inclusion in our study, 69% of which were male. Peak cortisol was inversely correlated with age (r= -03297, p= 0.0002). The median and 5th centile peak cortisol value were, respectively, 629 nmol/L (22.8 µg/dL) and 500 nmol/L (18 µg/dL) in children < 12 years as compared with, respectively, 564 nmol/L (20.4 µg/dL) and 457 nmol/L (16.5 µg/dL) in children ≥ 12 years. Median cortisol peak was significantly higher in younger patients compared to older patients (P=0.0004). **Conclusion:** The peak cortisol is age related. A single peak cortisol threshold in children of all ages is not appropriate and will result in overdiagnosis of adrenal insufficiency in adolescents.

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In Pursuit of an Optimal Vitamin D Assay in the Era of High Patient Volume and Complexity

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Background: The Institute of Medicine recommends total 25-hydroxyvitamin D (25-OHD) testing to screen high risk patients for deficiency and monitor supplementation therapy. Numerous factors (i.e. test volume, laboratory type and equipment, patient population) should be considered when determining the best methodology for 25-OHD testing. LC-MS/MS is the gold standard for measuring 25-OHD in the clinical laboratory, allowing for quantification of 25-OH D2/D3and respective epimers, however, the FDA only recommends reporting total 25-OHD. In practice, LC-MS/MS is manual, highly complex and time consuming. There are numerous 25-OHD immunoassays (IA), but historically these assays were subject to numerous interferences from a number of sources including 25-OHD metabolites and lipids. Herein, we describe a multicenter study comparing NIST standardized LC-MS/MS 25-OHD assays with three contemporary immunoassays in a complex patient population. **Objective:** To perform a multicenter study comparing two LC-MS/MS 25-OHD methods with three contemporary immunoassays in complex patient populations. **Methods:** 25-OHD in patient samples was quantified by three contemporary IA methods (Abbott Architect 25-OH Vitamin (New formulation), Roche Elecsys Vitamin D total II, and BioRad BioPlex 25-OH Vitamin D). Results were compared to one of two NIST standardized LC-MS/MS methods (which detect 25-OH-D2 (D2) and 25-OHD3 (D3)) at two clinical laboratories (University of Kentucky (UK) and/or Seattle Children's Hospital (SCH)). The 3-epi 25(OH)D (epimer) was chromatographically resolved by SCH-LC-MS/MS. Clinical information was obtained from patients' Electronic Health Record. Statistical analyses were performed in EP Evaluator. **Results:** The table shows a comparison of 25-OHD results between contemporary IA and LC-MS/MS methods in complex population and subpopulations. **Conclusion:** The 25-OHD results correlated well between LC-MS/MS and contemporary immunoassay methods in the total population; however, some subpopulations had poor correlation. Laboratories should consider the contribution of 25-OHD isomers, epimers and other minor metabolites when choosing a Vitamin D assay in complex patient populations.

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Molecular heterogeneity of Macroprolactin in samples suspected of false hyperprolactinemia.

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Background:
Circulating prolactin (PRL) exists in several molecular forms with different biological activities, in some instances making PRL assay and its diagnostic value unreliable. Macroprolactin (macro-PRL), a 150 kDa molecular weight (MW) form, is known to have low biological activity which may lead to misdiagnosis and inappropriate treatment in patients with suspected hyperprolactinemia. Polyethylene glycol precipitation (PEGP) is widely used to identify the presence of macro-PRL, but up to 20% of monomeric PRL is lost during this procedure and PEG itself can interfere with some PRL immunoassays. Additionally, PEGP has been reported to give false positive results for macro-PRL in patients with increased serum globulins (IgG myeloma and HIV patients). The aim of this study is to identify different PRL variants in samples suspected of macro-PRL using gel filtration chromatography (GFC).
Methods:
Twelve samples obtained from Nichols Institute (San Juan Capistrano, CA) with measured PRL concentrations prior to and following PEGP were analyzed using GFC analysis. Briefly, 100 uL of sample or protein markers (molecular weights ranging from 12.4 to 200 kDa) were applied to a Superdex 200 column (Pharma-

cia, Sweden). Forty fractions of 0.5 mL each were collected per patient sample at a flow rate of 0.4 mL/min using PBS containing 1% (w/w) bovine serum albumin as the mobile phase. Elution of marker proteins was detected by recording absorbance at 280 nm. Prolactin concentrations of all GFC fractions were measured using ELISA (R&D systems, Minneapolis) according to the manufacture's protocol.
Results:
A total of 12 samples were subjected to GFC analysis. Nine samples had high levels of total PRL (30.2 - 1469 ng/mL) whereas 3 samples had total PRL levels within the reference intervals (21.4 - 29.4 ng/mL). All samples positive for macro-PRL by PEGP analysis (n=5) exhibited high-molecular-weight PRL (>150kDa) and mid-molecular-weight PRL (30-150kDa), with one of the samples also exhibiting low-molecular-weight PRL (<30kDa). In macro-PRL negative samples by PEGP (n=5), all showed mid-molecular-weight PRL, one sample had high-molecular-weight PRL, and one sample exhibited low-molecular-weight PRL as well. Two samples within the PEGP indeterminate zone (60-40% recovery) had mid-molecular-weight PRL, one sample exhibited low-molecular-weight PRL, whereas the other sample exhibited high-molecular-weight in addition. PEGP failed to identify a small amount of high-molecular-weight PRL in one sample seen by GFC analysis.
Conclusion:
For the majority of our samples (11 out of 12), PEGP results agreed with GFC results in identifying macro-PRL. GFC analysis showed marked molecular heterogeneity for macro-PRL as defined by PEGP analysis. Mid-molecular weight-PRLs were present in every sample and both high-molecular weight-PRL and mid-MW-PRL had diverse patterns, but their clinical significance and physiological roles remained unclear. This is the first study to report molecular heterogeneity for macro-PRL.

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Can baseline cortisol predict short synacthen test response?

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Background
The short synacthen test (SST) is the dynamic function test most widely used to assess hypothalamic pituitary adrenal axis. It is possible that a single basal cortisol value can predict the response of this dynamic test. Our aim was to determine a morning baseline cortisol value that could predict SST response.
Methods
We conducted a retrospective analysis of short synacthen test results (using Advia Centaur XP/ Siemens) of samples received to the radioimmunoassay laboratory in the National Hospital of Sri Lanka from May 2017 to October 2017. Patients who were acutely ill or in intensive care and on glucocorticoid therapy were excluded and 98 patients remained for analysis. The SST was considered to have an inadequate response when 30 min cortisol level was below 550 nmol/L. ROC curve was generated to determine a predictive value of basal morning cortisol for failing SST.
Results
Seventy five patients had adrenal insufficiency and 23 patients were adrenal sufficient. ROC curve had a good overall predictive value (AUC - 0.814; 95% confidence Interval 0.715 - 0.914). Baseline cortisol level predicting failing the SST with 100 percent specificity was 132 nmol/L (sensitivity of 36%). All the patients with a basal cortisol level of 442 nmol/L or above had passed the test (Sensitivity - 100% and specificity - 27%). A basal cortisol value of 256 nmol/L was recognized to predict adrenal insufficiency with sensitivity of 90% and specificity of 67%.
Conclusion
A single value with a high specificity and sensitivity which can predict the outcome of SST cannot be defined. If morning basal cortisol level is either < 132 nmol/L or > 442 nmol/L, it is not necessary to perform SST and it would have prevented 58 (58.6%) SST. Therefore basal morning cortisol might help in avoiding unnecessary SST and provides a cost-effective approach.
Keywords
Baseline cortisol, short synacthen test, adrenal insufficiency
Background

A-211

Free 25 Hydroxy Vitamin D by LC-MS/MS: Reference Intervals in Healthy Adults and Observations in Pre-/Post-Menopausal Women

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Background: Serum 25-hydroxy vitamin D (25OHD) is widely used as a marker of vitamin D sufficiency. In circulation, 25OHD is tightly bound to proteins with less than 0.1% circulating in free form. It has been demonstrated that the majority of human cells respond to the free, rather than protein-bound, form of 25OHD. Therefore, measurement of free 25OHD (F25OHD) may be a relevant biomarker for assessing the physiologically active fraction of vitamin D.
Methods: We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for F25OHD, evaluated its performance and established reference intervals for healthy adults. Sample preparation for F25OHD was performed as follows: F25OHD (F25OHD2 and F25OHD3) were separated from the protein bound fraction using size exclusion based technique; stable isotope labeled internal standard was added to the samples, 25OHD was derivatized and analyzed by LC-MS/MS. Two mass transitions were monitored for F25OHD2, F25OHD3, and the internal standards; ratio of the mass transitions was used to confirm specificity. Concentrations of calcium (Ca) and parathyroid hormone (PTH) were determined using Cobas 8000 modular analyzer (Roche Diagnostics, Indianapolis); total 25OHD (T25OHD2 and T25OHD3) was measured using LC-MS/MS. The method was compared to a commercial F25OHD ELISA (Future Diagnostics, The Netherlands). Reference intervals were established using samples from 120 self-reported healthy adult volunteers (52 men, 68 women; age range 20-63 years; 111 Caucasian, 6 Hispanic, 3 Asian; 63% collected during summer/fall months, 37% collected during winter months). Mean age was 38.5 and 34.5 for men and women, respectively; number of samples by age group was: 41, 34, 27, and 18 (20-30, 31-40, 41-50, 51-63 years old, respectively); 55 samples were from premenopausal women (PW) and 13 samples were from postmenopausal/perimenopausal women (PPW). Concentrations of Ca, PTH, and (T25OHD2+T25OHD3) in the samples were within the corresponding reference intervals.
Results: For F25OHD2 and F25OHD3, the lower limit of quantitation was 0.005 ng/mL and total imprecision at concentrations above 0.01 ng/mL F25OHD was <15%. Nonparametric reference intervals for F25OHD and percent F25OHD were 0.024-0.080 ng/mL and 0.08-0.18%, respectively. Statistically significantly higher concentrations of T25OHD (p=0.0089) and F25OHD (p=0.049) were observed in samples collected during summer/fall than during winter. No statistically significant difference in F25OHD concentrations were observed between men and women or among the age groups. While PPW had higher concentrations of T25OHD than PW (p=0.07), no statistically significant difference was observed in the distribution of concentrations of F25OHD between PPW and PW (p=0.543). However, statistically significantly lower percent F25OHD was observed in PPW than in PW (p=0.033).
Conclusion: In summary, we developed a LC-MS/MS method for measurement of F25OHD and established reference intervals for F25OHD and percent F25OHD in healthy adults. Lower percent F25OHD observed in PPW is likely explained by higher concentrations of binding proteins in this population, and could be a contributing factor to the higher incidence of osteoporosis observed in postmenopausal women.

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Evaluation of the cobas c 513 analyzer for HbA1c assay

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Background: Hemoglobin A_{1c} (HbA_{1c}) is an essential biomarker for monitoring glycemic control in diabetic patients and is also used in the diagnosis of diabetes mellitus. Therefore, reliable and efficient methods are required for HbA_{1c} quantification. This study evaluated the analytical performance of the Tina-quant® HbA_{1c} third generation immunoassay on the **cobas c** 513 analyzer.
Methods: Precision was assessed according to Clinical and Laboratory Standards Institute EP05 guidelines, using quality controls (n=2) and patient samples (n=4), which were analyzed in duplicate twice a day for 21 days, on the **cobas c** 513 (throughput: 400 samples/hour). Method comparison was performed against two routinely used high-performance liquid chromatography (HPLC) analyzers (D-100 and Variant II, BioRad laboratories). Accuracy was evaluated against 8 external quality assurance samples (European Reference Laboratory for Glycohemoglobin) with IFCC-assigned

target values (31.4-99.2 mmol/mol). Analytical interference by bilirubin, triglycerides and common Hb variants (Hb AC, AD, AE, AS) on HbA_{1c} quantification was assessed. Data are reported for HbA_{1c} values in IFCC units (mmol/mol) unless stated. **Results:** The HbA_{1c} assay demonstrated good precision, with coefficients of variation (CV) lower than 1.13% and 1.73% for HbA_{1c} values expressed in NGSP (%) and IFCC units, respectively (**Table**). Good correlation of the HbA_{1c} assay was observed with both HPLC systems (D-100: y=0.951x +2.757, r=0.997, n=100; Variant II: y=0.997x + 1.904, r=0.998, n=100). The analysis of samples with IFCC-assigned values showed a good accuracy of the method; relative biases ranged from -0.2% to 3.4%. No interference by bilirubin (0-352 µmol/L, relative bias -1.4% to 1.6%), triglycerides (0-20.6 mmol/L, relative bias 4.0% to 0.8%) and common Hb variants was observed. **Conclusion:** The HbA_{1c} assay on the **cobas c** 513 analyzer demonstrated a good analytical performance, and is therefore suitable for routine use in clinical chemistry laboratories.

Precision of the HbA1c assay on the cobas c 513 analyzer					
Sample	Mean value	Repeatability (CV, %)	Between-run precision (CV, %)	Between-day precision (CV, %)	Intermediate precision (CV, %)
HbA1c values in %					
Sample 1	5.80	0.46	0.62	0.75	1.08
Sample 2	6.07	0.67	0.61	0.65	1.12
Sample 3	7.90	0.49	0.79	0.63	1.12
Sample 4	11.61	0.62	0.63	0.71	1.13
QC sample (low-level)	5.74	0.57	0.46	0.41	0.84
QC sample (high-level)	11.13	0.54	0.21	0.91	1.08
HbA1c values in mmol/mol					
Sample 1	39.9	0.74	0.98	1.21	1.73
Sample 2	42.9	1.03	0.95	1.01	1.73
Sample 3	62.9	0.69	1.09	0.87	1.55
Sample 4	103.5	0.77	0.78	0.87	1.40
QC sample (low-level)	39.2	0.90	0.71	0.68	1.34
QC sample (high-level)	98.2	0.68	0.25	1.14	1.35

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Three alternative makers of hyperglycemia for early detection of diabetes: glycated albumin, 1,5-anhydroglucitol, and fructosamine

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Background: Glycated albumin (GA), 1,5-anhydroglucitol (1,5-AG), and fructosamine have recently attracted considerable interest as markers of hyperglycemia. However, these alternative markers are not understood well enough yet for use in the clinic. In this study, we assessed their potential utility in the early identification of hyperglycemia. **Methods:** We conducted an analysis of 5,800 participants who underwent healthcare study between August 2013 and September 2014 and had no history of diagnosed diabetes mellitus (DM). All of the tests were performed at the laboratory department in the Kangbuk Samsung Hospital Total Healthcare Center in Seoul, Korea. Blood specimens were sampled from the antecubital vein after an 8-hour fast. Serum GA, 1,5-AG and fructosamine levels were measured by automatic chemistry analyzer (Modular P800; Roche Diagnostics, Tokyo, Japan) using a Lucica GA-L reagent (Asahi Kasei Pharma Co., Tokyo, Japan) based on the enzymatic method; Determiner L 1,5-AG reagent (Kyowa Medex, Tokyo, Japan) based on the colorimetric method; and Fructosamine reagent (Roche Diagnostics GmbH, Mannheim, Germany) based on a colorimetric method, respectively. We divided the study population into normal, pre-DM, and DM groups according to fasting blood glucose (FBG) and HbA1c levels. Among them, 77.0% of the participants had follow-up examinations before July 2017 and 100 participants were newly categorized as DM group. The area under the receiver operator characteristic (AUC-ROC) curves was calculated to determine the ability of three alternative markers to predict hyperglycemia. We then conducted multivariate analysis to estimate DM progression. **Results:** Participants in the DM and pre-DM groups were more likely to be older than those in the normal group, with mean ages of 49.4 ± 9.9 (range, 33-76) and 44.2 ± 9.3 (15-77) vs. 39.7 ± 7.8 (18-77) years, respectively. Mean levels of GA, 1,5-AG, and fructosamine were significantly different among the three groups. At the ROC analysis, estimated cut-off values of GA, 1,5-AG, and fructosamine for the DM group criteria with HbA1c≥6.5% and/or FBS≥126 mg/dL were 13.1%, 11.8 ug/mL and 253 umol/L with good AUC values; 0.849, 0.862, and 0.818. For the pre-DM and DM groups criteria with HbA1c≥5.7% and/or FBS≥100 mg/dL estimated cut-off values were 12.2%, 17.2 ug/mL and 243 umol/L but the AUCs were poor as 0.552, 0.605, and 0.609. On follow-up, 4.8%, 5.7%, and 5.8% of the highest risk quintile groups according to baseline levels of GA, fructosamine, and 1,5-AG had progressed from non-DM to DM group, while the lowest risk quintile of each group exhibited 1.4%, 0.6%, and 1.7% progression. The highest risk group of 1,5-AG showed a higher odds ratio(OR) for the DM progression than those of GA and fructosamine (estimated OR after adjustment for confounding variables: 4.720, 12.509 and 3.667 at the highest risk group of GA, 1,5-AG, and fructosamine). **Conclusion:** The highest risk quintile groups of these three markers were associated with progression to DM. Our results suggest that these markers may be useful alternatives and supporting to the traditional markers in early screening for hyperglycemia.

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Multicenter performance evaluation of the Elecsys® Insulin-like Growth FactorI immunoassay and establishment of reference ranges in a large cohort of healthy subjects

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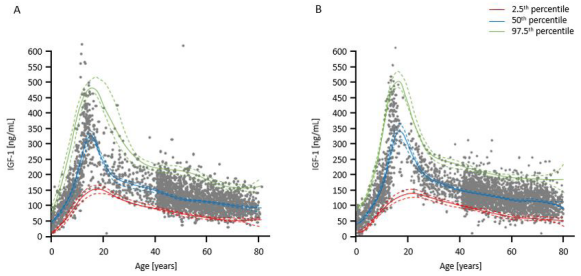
Background: Insulin-like growth factor-I (IGF-I) is a biomarker used to assess disorders of the growth hormone/IGF axis. We evaluated the analytical performance of the Elecsys® IGF-I electrochemiluminescence immunoassay and established reference ranges in apparently healthy subjects. **Methods:** Three laboratories evaluated the Elecsys IGF-I assay (Roche Diagnostics) under routine conditions. Experiments were performed on **cobas e** 411 and 601 analyzers. Repeatability (within-run) and intermediate precision (within-laboratory) were assessed according to Clinical Laboratory Standards Institute (CLSI) EP5-A3 guidelines (5-day model, 3 reagent lots [2 per site]). Samples comprised 7 human serum pools and 2 control samples (PreciControl Growth, Roche). Functional sensitivity was evaluated, and method comparisons of Elecsys IGF-I assay (measuring range: 7-1600ng/mL) versus 3 commercial assays were performed (CLSI EP9-A3 guidelines). Clinical evaluations used samples from the LIFE (NCT02550236) and EU Sample Collection studies. **Results:** Samples with moderate/high (55.2-1487ng/mL) and low IGF-I levels (22.2-25.9ng/mL) met predefined acceptance criteria for repeatability, intermediate precision and inter-module precision; CVs for moderate/high samples were 1.0-3.2%, 1.0-6.3% and 3.5-6.7%, after excluding outliers. Functional sensitivities for each lab (CV threshold 20%) were 4.75, 7.23 and 11.30ng/mL IGF-I, respectively. The Elecsys IGF-I assay showed good agreement with IGF-I results from IDS iSYS (Passing-Bablok regression slope, 1.13; intercept, -14.0; Pearson's r, 0.995; n=146), Siemens Immulite 2000 (0.873; -28.8; 0.956; n=135) and Diasorin Liaison (0.859; -14.0; 0.993; n=145). Evaluation of samples from 6698 apparently healthy subjects (age: 3 months-80 years) showed IGF-I concentrations for both sexes increased rapidly from birth, reaching a peak median concentration at 15 years in females (n=3046; 331ng/mL) and 17 years in males (n=3652; 340ng/mL); levels decreased sharply during early adulthood and remained relatively constant in senescence (**Figure**). **Conclusion:** We demonstrate robust analytical performance of the Elecsys IGF-I assay under routine conditions and provide gender-dependent reference ranges based on results from apparently healthy subjects.

Figure. Quantile regression of IGF-I serum concentrations in apparently healthy **(A)** females (n=3046) and **(B)** males (n=3652). Plotted curves represent 2.5th, 50th and 97.5th percentiles (solid lines) and associated 95% confidence intervals (dashed lines).



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Fractional Excretion of Vitamin D Binding Protein as a Novel Marker of Incipient Diabetic Nephropathy and Vitamin D Status in Subjects with Type 2 Diabetes Mellitus

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Background: Vitamin D deficiency (VDD) has been shown to play significant roles in the pathogenesis and complications of Type 2 Diabetes mellitus (T2DM). Vitamin D-binding protein (VDBP), a 58-kDa glycoprotein, is a significant determinant of biologically active levels of 25(OH) Vitamin D (25(OH)D). Studies have shown increased urine excretion of VDBP in patients with diabetic nephropathy (DN) but the exact mechanism of increased VDBP excretion is not clearly understood. The endocytotic receptor pathway in renal tubules is involved in the reabsorption of 25(OH)D and VDBP filtered in the glomerulus and, with the onset of nephropathy in diabetes, we hypothesized that increased urine protein load could affect clearance of VDBP. In this study, we evaluate the utility of the Fractional Excretion of VDBP (FEVDBP) as a novel index of VDD and DN. **Methods:** Levels of 25(OH)D, HbA1c, serum and urine concentrations of VDBP, creatinine were measured in 405 (129M, 276F) T2DM patients. Ratio of urine microalbumin to creatinine was determined to categorize subjects as normoalbuminuric (NAO, ratio <30mg/g); microalbuminuric (MIA, ratio 30-300mg/g) and macroalbiminuric (MAA, ratio >300 mg/g). FEVDBP was calculated as 100 x (UrineVDBP x SerumCreat) / (SerumVDBP x UrineCreat). Univariate and multivariate analyses were used to compare study subjects grouped by Vitamin D status, glycemic control and degree of microalbuminuria**.** **Results:** VDD (<50nmol/L; n = 237) or insufficiency (VI) (50-75 nmol/L; n=84) was prevalent. Urine VDBP concentration increased stepwise with increasing degrees of microalbuminuria. Mean FEVDBP in subjects with normal 25(OH) D, VI and VDD were 5.5, 5.4 and 8.5 respectively; mean FEVDBP in NAO, MIA and MAA were 3.7, 23.3 and 55.9 respectively. Significant correlations of FEVDBP were with age (r=0.38), glucose (r=0.42), HbA1C (r=0.46), urine microalbumin:creatinine ratio (r=0.56) and significant negative correlation with serum albumin (r = - 0.30). Receiver operating Curve (ROC) analyses of the use of FEVDBP for detection of VDD, microalbuminuria and poor glycemic control showed that the areas under the ROC are 0.545, 0.822 and 0.732 respectively. **Conclusion:** Unlike other studies where only urinary concentrations of VDBP was evaluated, we assessed the ratio of VDBP excreted in the urine taking into account its levels in the plasma thereby providing a more accurate measure of filtered VDBP. Increased FEVDBP in MIA and MAA confirms our hypothesis that onset of nephropathy in diabetes increases the clearance of VDBP. We conclude that increased FEVDBP contributes to the mechanisms of VDD in T2DM. The significant associations of FEVDBP with glycemic control and DN suggests that this index could play a wider role in the pathogenesis and/or detection of diabetic complications.

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TSH immunoassays: Commutability of EQAS samples and relation to inter-method differences

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Background: Selectivity and reference preparations are the basics of a traceability chain for measurands with no reference measurement system available. All immunoassays claim to measure hTSH and use the same WHO IRP, they should produce comparable results. In a method comparison study of the IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT), 13 out 16 hTSH immunoassays had their standardization set-point within 10% from the all-procedure trimmed mean, however, the average dispersion across all assays was of the order of ~32%. Same results can be observed from EQAS results. Commutability of samples can be also an issue in intermethod differences. The aim of this study was to investigate different start-up materials for EQA samples and the relationship with differences observed in TSH results from EQAS Buenos Aires (ProgBA). The EQAS is accredited under ISO/IEC 17043:2010, and TSH is included in the scope. **Methods:** Freeze-dried human samples were distributed in 2017 to 200 participants in Latin America. Major IVD assays were IMMULITE SIEMENS, CENTAUR SIEMENS, ACCESS BECKMAN, COBAS ROCHE, and ARCHITECT ABBOTT, all of them calibrated against WHO IRP 80/558. Start-up materials were pooled sera or plasma from single donor coagulated with thrombin. Statistics were calculated following ISO 13528: 2015. All laboratories´ trimmed mean (ALTM) was used for comparison. **Results:** For low TSH level, ALTM=0.48 mUI/L, %differences ranged -10.03 to 15.7, span between lowest (COBAS) and highest (ACCESS) was 36%. For normal TSH levels, pooled serum ALTM=1.59 mUI/L %differences ranged -9.2 to 7.1, span between lowest (IMMULITE) to highest (COBAS) 16%; plasma ALTM 2.24 mUI/L %differences -12.6 to 5.1, span lowest (ARCHITECT) to highest (COBAS) 17%. For TSH level 4 mUI/L, plasma ALTM 4.41 mUI/L %differences -3.99 to 6.79, span lowest (ARCHITECT) to highest (CENTAUR) 11%. **Conclusion:** Results from EQAS showed same differences as those in the WG-STFT study, suggesting that differences observed are not defined by commutability of materials but by different assay standardization. Also these results support the role of EQAS as an important actor in IVD standardization / homogenization for hormone immunoassays.

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New assay for testing anti-TSH receptor antibodies: is TSI comparable to other methods available?

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Background: Autoimmune thyroid illness (ATI) accounts for a number of disorders as hypothyroidism, hyperthyroidism and goiter. ATI is associated to autoantibodies against antigens like thyroid-stimulating hormone receptor (TSHR). Two types of TSHR antibodies are involved in the physiopathology of ATIs: 1) thyroid stimulating immunoglobulins (TSI) are present in Graves' disease (GD); 2) blocking antibodies prevent binding of TSH to its receptor (TRAb-B) and can lead to hypothyroidism in Hashimoto´ disease. Recently a third type was descripted, C-TRAb (Apoptotic), with biological activity, responsible together with cytotoxic Tcells specific for thyroid gland, of thyrocytes´ autoimmune depletion.Methods for TRAb detection include biological assays (BAs) and immunoassays (IAs). BAs measure TRAb activity while IAs measure antibody binding to receptor, without discrimination on function (totalTRAb). A new automated IA claims to detect only TSI, which would give high sensitivity in detecting GD, and high specificity for discriminating between GD and other ATIs. The aims of this work are the evaluation of performance of this new method for TSI and to compare it with other IAs available locally for TRAb testing. **Methods:** Immunoassays compared were TSI Immulite® (Siemens Healthcare), cutoff 0.55 IU/L calibration 2nd IS (NIBSC 08/204); TRAb% RSR® Limited (UK): cutoff 15%, calibration CRM LATS standard B; and TRAb-Cobas (Roche Diagnostics): cutoff 1.75 IU/L, calibration 1st IS (NIBSC 90/672). 290 negative, mild positive and strong positive serum samples from patients aged 6 to 82, 80% female, were included in the comparison: 148 samples were selected previously tested by RSR method and 142 by Cobas. 98 samples were tested by the three methods and the ratio of positive sample/cutoff (RP) was calculated for each method. **Results:** Comparing RSR and TSI (n=148), 24% samples were negative by both methods, 11.4% were discordant, and for those classified as positive by both methods, important differences were observed in RP values; when comparing TRAb-Cobas with TSI (n=142), 33% samples were negative by

the two methods, 7.7% samples were discordant, and for those classified as positive by the two methods, also important differences were observed in the RP values. For the 98 samples processed by the 3 methods, those showing values of 16-30% in RSR, in Cobas were between 0.90 y 18.87 IU/L and in TSI from 0.10 to 13.6 IU/L. Significant differences in the absolute values of RSR vs TSI and Cobas vs TSI were obtained (*p* less than 0.001, Wilcoxon signed Rank test, paired samples). Same differences were obtained comparing only positive samples in Cobas vs TSI (n: 89). Also, significant differences in RP values between RSR vs TSI and RSR vs Cobas (*p* less than 0.001) were obtained, but no significant difference was obtained between RP from TSI vs RP from Cobas (Friedman-Dunn test). **Conclusion:** Although the new Immulite method claims to be more specific for detection of TSI, the %discordances actually observed do not support this affirmation, as RP values do not correlate. We conclude that more data are needed to establish the real clinical usefulness of this new assay.

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Performance evaluation of two fully-automated anti-Müllerian hormone assays and comparison against current manual enzyme-linked immunosorbent assay

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Background: Anti-Müllerian hormone (AMH), a dimeric glycoprotein produced by ovarian granulosa cells of pre-antral and small antral follicles, is a reliable serum biomarker to assess growing ovarian pool. AMH test is increasingly being used as a surrogate biomarker for ovarian reserve tests (ORTs) and recombinant follicle-stimulating hormone (rFSH) dosing in infertility clinics. However, its routine clinical use is limited by the low throughput and a high degree of inter-laboratory variability of enzyme-linked immunosorbent assays (ELISA). The authors evaluated the performance of two new fully automated AMH immunoassays and compared them with a currently used AMH ELISA method. **Methods:** Two fully-automated AMH assays with electro-chemiluminescence immunoassay (ECLIA) platform using the Roche cobas e602 analyzer (Roche Diagnostics GmbH, Germany) and chemiluminescence immunoassay (CLIA) platform using the UniCel DxI automated analyzer (Beckman Coulter Inc., USA) were compared to a current ELISA assay (AMH Gen II ELISA, Beckman Coulter, USA). Precision analysis (according to CLSI EP5 guideline), repeatability and linearity (according to CLSI EP6 guideline) were assessed for both of the two automated immunoassays. Anonymized remnant 113 serum samples from routine AMH ELISA testing were used for comparing the two automated immunoassays against the ELISA assay. **Results:** Both of the two automated AMH assays showed excellent precision, repeatability and linearity. The total coefficient variation (CV) of Beckman CLIA and Roche ECLIA assays were 3.5 - 4.1 % and 2.4 - 3.6 %, respectively, over a range of concentrations. AMH concentrations measured with the Beckman CLIA showed better correlation when compared with the AMH Gen II ELISA than the Roche ECLIA (CLIA = -0.149 + 0.877 ELISA, R = 0.983; ECLIA = 0.172 + 0.667 ELISA, R = 0.932). Both of the two automated assays showed significant negative bias when compared with the Gen II ELISA (mean % difference; -20.33% between ELISA and ECLIA vs. -12.17% between ELISA and CLIA). **Conclusion:** The two automated AMH immunoassays showed advantages including high throughput and superior analytical performance over the current Gen II ELISA platform. However, considerable degrees of systematic difference were noted between the automated immunoassays and the Gen II ELISA, with larger negative bias in case of the Roche ECLIA platform. Automated AMH assays need to be harmonized to the conventional ELISA to adapt currently using clinical cut-offs which is mostly based on the Gen II ELISA format.

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The comparison of cortisol level by electrochemiluminescence Immunoassay radioimmunoassay and mass spectrometry

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Background: Cortisol is the main glucocorticoid of the adrenal cortex, regulating energy metabolism, blood pressure, stress responses, etc. To diagnose cortisol related disease such as Cushing's syndrome or Addison's disease, it is important to provide accurate level of cortisol. In Korea, current practice to measure cortisol level is to use electrochemiluminescence Immunoassay(ECLIA) and radioimmunoassay(RIA). Immunoassays have problems with varying selectivity and specificity. As for cortisol, there are many endogenous metabolites and drugs that interfere with immunoassay. In this study, we compared HPLC-MS/MS with ECLIA and RIA to measure serum cortisol level. **Methods:** Cortisol levels from 28 samples referred to Seoul Clinical Laboratories(SCL) in Korea were measured by MS/MS(AB Sciex, CA, USA), ECLIA and RIA. 200ul samples, control, and calibrator with cortisol-9,11,12,12-d4 were mixed with ethyl acetate 2mL. After 30 seconds of vortexing, centrifugation for 5 minutes at 4000 rpm was performed. Obtained supernatant was placed under, N2 gas for evaporation. Reconstruction was done with 100uL of 30% methanol. 20 uL of sample and control were placed on auto-sampler. Separation was carried out on Imtakt Unison UK C18 column(2.0 x 50 mm, 3um) followed by MS detection using an ABSciex 5500 mass spectrometer. Detection was performed in the positive electrospray multiple reaction monitoring (MRM) quantitation. ECLIA of cortisol was performed on the Cobas e601(Roche, Grenzacherstrasse, Switzerland). The RIA(Immunotech s.r.o, Prague, Czech) was performed according to the manufacturer's instructions. Samples and calibrators are incubated with an 125I-labeled cortisol, as tracer, in antibody-coated tubes. Results were compared by ANOVA, linear regression analysis and using the methods described by Bland and Altman. **Results:**
• Cortisol levels by MS/MS, ECLIA and RIA ranged from 1.35 to 48.8 (median 20.98) ug/dL, from 0.99 to 55.2 (median 21.18)ug/dL and from 1.20 to 68.3 (median 23.89)ug/dL, respectively. ANOVA analysis concluded three methods do not show difference. • Numbers of samples higher than the reference value by MS/MS, ECLIA and RIA were 11, 12 and 11, respectively. • Numbers of samples lower than the reference value by MS/MS, ECLIA and RIA were 4, 6 and 3, respectively. • When comparing MS/MS with ECLIA, linear regression analysis revealed the following equation: ECLIA = 1.0999xMS/MS - 1.8929 (R^2 = 0.9821). • When comparing MS/MS with RIA, linear regression analysis revealed the following equation: RIA = 1.2677xMS/MS - 2.7024 (R^2 = 0.8891). **Conclusion:** Although ANOVA analysis concluded three methods do not show difference, correlation between RIA and MS/MS was not as good as ECLIA and MS/MS. The difference between RIA and MS/MS gets bigger as the level of cortisol goes higher, which becomes as big as -22.10ug/dL. The external quality controls of three methods have been within acceptable ranges. Therefore, it is recommended for clinicians to recognize different method can show different result. Furthermore, there should be international effort to develop certified reference materials(CRM) and accuracy based proficiency test to standardize the measurement of cortisol.

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Development of the ARCHITECT Tg Assay for Quantitation of Human Thyroglobulin

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INTRODUCTION: Thyroglobulin (Tg) is a protein that is readily secreted by the thyroid gland. Tg is primarily used as an aid in monitoring after total thyroid ablation (thyroidectomy). **METHODS:** The ARCHITECT Tg assay (in development) is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative measurement of Tg in human serum or plasma on the ARCHITECT *i* System. This method utilizes paramagnetic microparticles coated with a highly specific monoclonal antibody (mAb), which captures Tg present in the specimen. After incubation, the acridinium-labeled anti-Tg mAb conjugate is added to complete the sandwich. After another incubation and wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of Tg present in the specimen and the RLUs detected by the ARCHITECT *i* System optics. **RESULTS:** The ARCHITECT Tg assay is traceable to the European Community Bureau of Reference (BCR) CRM 457. Across three lots, the ARCHITECT Tg assay demonstrated linearity from 0.05-673.72 ng/ml. The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the ARCHITECT Tg assay were 0.06 ng/mL, 0.10, ng/ml. and 0.12 ng/ml, respectively. A twenty-day precision study of 3 controls and 5 panels assayed twice per day (n=80 for each sample) demonstrated within laboratory (total) precision of ≤ 5.5% for controls and ≤ 4.9% for panels. A method comparison of the ARCHITECT Tg assay with a predicate device was performed using Passing-Bablok regression resulted in a 0.99 slope, 0.31 ng/mL y-intercept, and 0.995 correlation coefficient (r) across the assay range of 0 - 500 ng/mL. In the range of 0 -10 ng/mL, Passing-Bablok regression resulted in a 1.06 slope, 0.00 ng/mL intercept, and 0.97 correlation. A tube-type equivalence study was performed using 6 non-primary tubes (Plasma K2 EDTA, Plasma K3 EDTA, Sodium EDTA, Serum Separator, Plasma Lithium Heparin, and Plasma Separator) compared directly to a primary Serum red-top with observed slopes of 0.96-1.01. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfer-

ing compounds, including 7 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum album, human anti-mouse antibody, and rheumatoid factor) and 14 commonly used therapeutic drugs (aminodarone HCL, carbimazole, D-T3, hydrocortisone, iodide, L-T3, L-T4, octreotide, potassium percholorate, prednisolone, popanolol HCL, propylthiouracil, thiamazole, fluocortolone pivalate). Cross-reactivity of the ARCHITECT Tg assay with Thyroid-Stimulating Hormone (TSH, 1000 mIU/L) and Thyroxine-Binding Globulin (TBG, 200,000 ng/ml) that are both similar in structure to Tg demonstrated no detectable cross-reactivity (0%). There was no high-dose hook effect observed for samples containing up to ~100,000 ng/ml of Tg. The ARCHITECT Tg reagents demonstrated on board stability and calibration stability on the instrument for a up to 30 days. **CONCLUSIONS:** These data demonstrate that the ARCHITECT Tg assay is sensitive, accurate and precise for the quantitative determination of Tg in serum and plasma specimens.

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Performance of the Diazyme Laboratories, Inc Glycated Serum Protein (Glycated Albumin) Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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Background: The Diazyme Laboratories, Inc. glycated serum protein (GSP) assay quantitatively determines the concentration of glycated proteins (Glycated Albumin) in serum. GSP is formed by a non-enzymatic Maillard reaction between glucose and amino acid residues of proteins. In diabetic patients, glycated serum proteins are a medium term indicator of diabetic control (2-3 weeks). GSP can be used in patients with conditions that interfere with RBC lifespan which may reduce the reliability of HbA1c measurements. Traditional fructosamine is also a glycated protein based assay but it utilizes a nitroblue tetrazolium (NBT) method, which in addition to glycated proteins reacts with various endogenous reducing substances such as thiol groups, NADH, and ascorbate. Studies showed that only about half of the reducing activity (Fructosamine) was due to glycation of proteins, and the remaining unspecific activity varied from serum to serum. The mechanism for the Diazyme GSP assay is that GSP is enzymatically digested and a proprietary amadoriase is used to catalyze the oxidative degradation of Amadori product protein and amino acids to glucosone and H_2O_2. The H_2O_2 released is measured by a colorimetric Trinder end-point reaction at 540 nm which is proportional to the concentration of glycated serum proteins. **Methods:** . The performance of the Diazyme GSP assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System was assessed on the VITROS MicroTip assay processing side of the MicroImmunoassay Center using 7.5 uL of patient sample and the Diazyme GSP reagents. Endpoint absorbance measurements were converted to a concentration using a linear calibration model. **Results:** The accuracy of Diazyme GSP assay was evaluated with 65 serum patient samples (134 - 1230 µmol/L) on the VITROS 4600 and VITROS 5600 Systems then compared to the predicate device Roche Hitachi 917 following CLSI: EP9-A2 guidelines. The VITROS 4600 and VITROS 5600 System showed excellent correlation with the Roche Hitachi 917. VITROS 4600 System R^2 value of 0.9946 with a slope= 1.0024, y-intercept of -4.77 and % bias of ≤ 10%. VITROS 5600 System R^2 value of 0.9911 with a slope= 1.0182, y-intercept of -3.07 and % bias of ≤ 10%. A 20-day precision study conducted on the VITROS 4600 and VITROS 5600 Systems at mean GSP concentrations of 251 µmol/L and 743 µmol/L resulted in within-laboratory percent coefficient of variation (%CV) of 3.76 % and 3.11% respectively, for the VITROS 4600 System and 2.40% and 1.55% respectively, for the VITROS 5600 System. The Limit of Quantification (LoQ) check for the VITROS 4600 and VITROS 5600 Systems was found to be ≤15.0 µmol/L. At 300 µmol/L common interfering endogenous substances of ascorbic acid 5 mg/dL, bilirubin 7.5 mg/dL, conjugated bilirubin 5 mg/dL, hemoglobin 200 mg/dL and triglycerides 2000 mg/dL showed no significant interference (≤ 10%). **Conclusion:** The Diazyme Glycated Serum Protein assay run on the VITROS 4600 and VITROS 5600 Systems demonstrated excellent correlation with the Roche Hitachi 917 Clinical Chemistry Analyzer, exceptional precision, and low-end sensitivity. Additionally, the assay was free from interference by endogenous substances at clinically relevant GSP concentrations.

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Dihydrotestosterone (DHT) Quantification in Blood Serum by LC-MS/MS after Derivatization for Research Purposes

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Background: Reduction of testosterone by 5α-reductase yields dihydrotestosterone (DHT), which is a more potent androgen than testosterone. Researchers studying the physiology of DHT and control of its biosynthesis using 5α-reductase inhibitors need to quantify this steroid within a range of 25 to 2000 pg/mL (2.5 - 200 ng/dL or 0.09 to 6.88 nmol/L) in blood serum. Since DHT does not ionize well by either atmospheric-pressure chemical ionization (APCI) or electrospray ionization (ESI), derivatization with hydroxylamine prior to liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was necessary to reliably achieve the desired measuring range. **Methods:** DHT was measured in donor blood serum samples using a multi-channel LC system coupled to a triple-quadrupole mass spectrometer with heated ESI source. 200 µL aliquots of specimens (calibrators, quality controls and donor serum samples) were spiked with DHT-D3 internal standard (IS) and then extracted by 2 mL of methyl-tert-butyl ether. The extracts were evaporated and the residues were reacted with hydroxylamine to form positive-ion oxime derivatives. The preparations were dried and reconstituted with 200 µL of water and methanol (1:1). 50 µL injections were made into heated 100 x 2.1 mm columns packed with solid-core silica particles with C18 and polar end caps bonded to available surfaces. A 4.5-minute mobile phase gradient from 50% methanol in water containing 0.1% formic acid to 100% methanol separated and eluted DHT and IS into the heated ESI source of the MS/MS system. Selected-reaction monitoring (SRM) of two transitions (quantitation and conformation) for DHT and IS occurred within a 2-minute data window. **Results:** Confirmation/quantitation ion ratios among calibrators, QCs, and specimens were within 20% of averages calculated from the calibrators. Intra-and inter-batch precisions among 20 replicate injections from three pools (low, medium and high DHT levels) were less than 7%CV. Carryover never exceeded 0.2%. Specimen IS peak areas averaged 70% relative to the averaged IS peak areas in calibrators and QCs, indicating moderate ion-suppression by matrix. However, the IS in each sample adequately compensated for matrix effects. The desired measuring range from 25 to 2000 pg/mL was achieved and was consistently linear ($r2 \geq 0.995$ with 1/X weighting). 54 donor samples were analyzed and results were compared with those from a reference lab. DHT values ranged from 47 to 973 pg/mL. The two DHT methods were equivalent within an allowable total error (TEa) of 25%. Only 4 out of 54 results differed by more than 20% and none were more than 22.4%. The differences between the two methods averaged 2.5%, which is a small positive bias. **Conclusion:** Derivatization with hydroxylamine prior to LC-MS/MS permitted reliable quantification of DHT in serum between 25-2000 pg/mL at a throughput of 13 injections/hour on a single LC channel. Throughput doubled by using an additional LC channel.

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Causes of Calculation Error Messages Encountered During the Measurement of HbA1c Using Cobas c502 Tina-quant® Assay

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Background: The Tina-quant Hemoglobin A1c (HbA1c) assay is a test with high specificity that can be used to diagnose diabetes, identify people at risk of developing the disease and ongoing monitoring. The aim of this study was to evaluate the possible causes of calculation error messages (CEMs) encountered during the measurement of HbA1c using this assay. **Methods:** All HbA1c samples performed over a period of one month, using Cobas c502 turbidimetric inhibition immunoassay (Tina-quant HbA1c assay), and had CEMs were investigated according to a special flow chart for HbA1c flags constructed by our laboratory according to manufacturer's recommendations. The error messages were divided into 4 categories; HbA1c less than the detection limit (A1 < test), HB less than the detection limit (HB < test), HbA1c more than the detection limit (A1 > test) and HB more than the detection limit (HB > test). Samples with CEM were checked for the presence of clots, remixed properly by inversion 20 times and reran on Cobas c502. Samples that gave a CEM of error >test after remixing and repeating were retested after dilution with 0.9% saline. Hematology samples, collected on the same day and at the same time, were used to double check and/or confirm the results of some of the samples with CEM. **Results:** out of 17,600 HBA1c samples, 177 (1%) were associated with CEMs; 120 (67.4%) had A1 < test, 72 (40.4%) had HB < test, 54 (30.3%) had A1 > test and 13

(7.3%) had HB > test. Evaluation by applying our flow chart for HbA1c flags revealed the association between theses samples and different conditions; 116 (65.5%) had low or high CEMs and corrected after remixing, 3 (1.7%) had low CEMs with normal HB and did not correct after remixing, 15 (8.5%) had low CEMs with low HB and were cancelled, 7 (4.0%) had high CEMs and gave exact readings after dilution, 30 (16.9%) clotted samples and were cancelled and 6 (3.4%) which were kept at room temperature for long time, had high CEMs, but refrigerated hematology samples of the same patients directly gave exact readings without the need for dilution. Total number of canceled results was only 33 (18.6%) of the samples with CEMs. **Conclusion:** Majority (83.4%) of CEMs are caused by preanalytical errors, which can be effectively prevented by simple techniques such as proper mixing and refrigeration of samples.

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Macroprolactin is not predicted by prolactin concentrations greater than 100 ug/L above validated prolactin reference intervals

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Objective Confirmation of current prolactin reference intervals in male and female populations, and identify their application in defining reflex testing rules for macroprolactin assessment in community and tertiary care patients. **Relevance** Prolactin is released from the anterior pituitary; it is the principle hormone controlling lactation initiation and maintenance. Hypo- and hyperprolactinemia may be associated with endocrine disorders, and hyperprolactinemia may also occur from prolactin secreting pituitary adenomas. Differential diagnosis of these conditions relies upon suitable prolactin reference interval, while also ruling out macroprolactin in asymptomatic patients. Macroprolactin is a prolactin complex bound to immunoglobulins, it is biologically inactive, and has a prolonged half-life versus monomeric prolactin. The macroprolactin complex is immunoreactive and can interfere in prolactin measurement, resulting in high prolactin concentrations despite asymptomatic patient presentation. **Methodology** Prolactin and macroprolactin results were compiled from community and tertiary care patients in Calgary, AB between January 1, 2015 and March 31, 2017 (*N*=65,561). Prolactin concentrations from plasma and serum specimens were measured using a sandwich immunoassay with two monoclonal antibodies against human prolactin and electrochemiluminescent detection (Roche Cobas 8000 e602). Macroprolactin testing was completed on all patients with prolactin concentrations above the upper reference limit (URL; 15 ng/mL men; 25 ng/mL women), with diagnosis of macroprolactin based upon prolactin recovery in polyethylene glycol (PEG) treated vs untreated specimens. A macroprolactin result with ≤40% recovery indicates macroprolactin (40-60% indeterminate). Prolactin reference intervals in both male and female populations were assessed by: 1) Bhattacharya analysis (*N*=15,529), 2) measurement in random specimens (*N*=60), and 3) measurement in healthy volunteers (*N*=20). Bhattacharya analysis and correlation assessment between macroprolactin incidence and prolactin concentration was performed in Microsoft Excel 2010 and IBM SPSS Statistics 19. **Results** Over approximately 2 years, macroprolactin testing increased (6.8-fold males; 1.8-fold females), while prolactin testing increased by 11.2% in males and decreased by 12.3% in females. Reference intervals were established (4-25 ng/mL females; 4-15 ng/mL males) following measurement in healthy volunteers. Positivity for macroprolactin was 4% in males and 7% in females, while indeterminate result was found in 4% of males and 10% of females. Prolactin concentration was identified as a poor predictor of macroprolactin detection by PEG precipitation; restricting macroprolactin measurement in specimens below the upper limit of the prolactin reference interval is a better predictor. In addition, prolactin results >100ng/mL above the URL (>116 µg/L males; >126 µg/L females) should not undergo macroprolactin investigation, as they have 0% positive predictive value for macroprolactin (N=569). **Conclusions** Prolactin reference intervals can only be determined within correctly identified healthy populations due to significant variability and preanalytical influences. Prolactin concentration was a poor predictor of macroprolactin findings; macroprolactin was not found in any specimens with prolactin concentrations >100 µg/L above the upper reference interval.

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Performance Evaluation of a Coupled Enzymatic Glycated Albumin Assay in Serum and Plasma Samples

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Background: Glycated albumin is a plasma protein formed by the nonenzymatic reaction of glucose and albumin. It has utility in monitoring glucose control in patients for whom hemoglobin A1c is considered unreliable. This includes those in whom the erythrocyte life span is altered, patients undergoing dialysis for end stage renal disease, or women during pregnancy. Additionally, it provides information on short-term (2-4 weeks) glycemic control that could be used prognostically. Although widely used in Japan, FDA-approved tests for this analyte are lacking in the U.S. We thus examined the performance of a commercially-available glycated serum protein assay which utilizes a coupled enzymatic assay adaptable for use on a variety of automated chemistry analyzers. **Methods:** Samples used in evaluation were either serum or plasma (heparin- or EDTA-containing tubes). Glycated Serum Protein (µmol/L, GSP) was measured using a coupled enzymatic assay (Diazyme, Poway, CA) on a Roche Cobas c501 automated platform. Albumin was measured using the Roche bromocresol green assay on the same platform. Percent glycated albumin (% GA) was calculated from GSP and albumin concentrations using the equation recommended by the manufacturer. Multi-day and within-day imprecision were determined using percent coefficient of variation (%CV). Accuracy was examined by comparison to certified reference material (JCCRM611-1) and samples obtained from the College of American Pathologists Proficiency Testing (CAP-PT) Survey. Linearity was examined using eight non-zero points in duplicate. **Results:** For serum, typical within-day %CV was 1.6% at both 13.0% and 50.2% GA. For heparin plasma samples, within-day %CV was 2.3% at 18.8% GA and 1.7% at 13.3% GA. %CV was slightly higher for the GSP measurement alone. Between-day precision (7 days, triplicates) was 2.3% for 13.6% GA in serum; in heparin plasma, it was 2.5% for 15.0% GA and 3.3% for 26.7% GA. Percent recovery compared to JCCRM611-1 was 103-104% at 12.8% and 20.2% GA. Results also fell within the expected range compared to peer results from the CAP-PT Survey. Linearity and lower limit of quantitation showed acceptable results for the GSP range of 86 -946 µmol/L (m= 1.014, b= -1.65, standard error of the estimate=14.74). With an albumin of 4.4 g/dL this range would correspond to 7.0 - 43.0% GA. A previously published reference interval of 10.5 - 17.5% (J Diabetes Sci Technol 2015; 9:192-9) was verified using 50 patient samples (25 female, median age 43 years, HbA1c ≤5.7), with only one outlier. Serum samples at room temperature were stable for 6 hours (<1.1% difference). Plasma samples collected in heparin tubes showed <4% difference in results under these conditions. Both matrix types showed stability to 7 days at 2-8 °C, but were no longer stable at day 14. Samples collected in EDTA showed instability during the same conditions. **Conclusion:** This assay for %GA shows very good precision and accuracy at low and high concentrations of analyte. The linear measuring range as determined for GSP, when using an average albumin concentration, corresponds to 7.0% - 43.2% GA, which would be useful for diabetic patients. Both heparin-containing and serum tubes are acceptable.

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Creating highly accurate and precise measurements of free thyroxine (T4) for the CDC Clinical Standardization Programs

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Background: Free thyroxine (FT4) measurements are used to diagnose and treat thyroid disorders, such as Graves' disease, Hashimoto's thyroiditis, and thyroid cancer. Reliable FT4 measurements are essential for assessing thyroid function and properly diagnosing and treating thyroid disorders. Although FT4 measurements are used extensively in research and clinical settings, the accuracy and reliability of current methods prevent proper detection, treatment, and prevention of thyroid disorders in patient care, making standardization of FT4 measurements a priority. Currently, there are no serum-based reference materials commercially available for FT4. CDC is working with the Committee for Standardization of Thyroid Function Tests (C-STFT) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to address these issues through development of an accurate and sensitive higher-order Reference Measurement Procedure (RMP) for FT4 as part of IFCC C-STFT and CDC's Clinical Standardization Programs. **Methods:** FT4 was measured using a modified equilibrium dialysis procedure.[1] FT4 in serum was isolated from the binding proteins in 1 mL PTFE equilibrium dialysis cells for 4 hours at 37°C. Dialysates were spiked with internal standard (thyroxine-13C6) and purified by C18 solid phase extraction (SPE) prior to injection on a Shimadzu UHPLC system coupled with an AB Sciex 5500 triple quadrupole mass spectrometer equipped with a TurboV electrospray ionization source. Bracketed calibration and primary reference materials were used to determine concentration of FT4 in serum. Chromatographic separation was achieved using a C18 reverse phase column with a gradient of water and acetonitrile with addition of 0.1% formic acid. Quantification by selective reaction monitoring (SRM) analysis was performed in the positive ion mode. Two transitions were monitored for each analyte and internal standard, and triplicate injections were used to minimize any instrument instability. **Results:** The proposed RMP has been evaluated and optimized for precision, accuracy, and sensitivity. The within-day and between-day impreci-

sion of 2.2-3.9% and 1.8-2.6%, respectively, were determined using CLSI EP10. By comparisons with the Reference Laboratory at the University of Ghent, the proposed CDC RMP reported a bias within +1.0%. Maximum extraction of the analyte prior to injection on the mass spectrometer is critical to ensure the sensitivity of the method is adequate. For example, it was determined that a 55% loss in signal could occur during sample preparation if the sample came into contact with either PTFE-lined or rubber caps, and a 16.5% loss in signal could occur with the use of plastic wellplates versus glass LC/MS vials before analysis. Taking into account these key factors, the limit of detection using 1.0 mL of serum was 0.312 pg on-column (0.126 ng/dL). **Conclusion:** To ensure accurate values are reported for FT4, careful consideration is needed for all steps of sample preparation and analysis. This candidate reference method for FT4 in serum demonstrates good accuracy and precision, and as such this method can be used as a viable base for accuracy to which routine methods for FT4 can be compared. [1]Van Houcke, A.K., et. al. IFCC international conventional reference procedure for the measurement of free thyroxine in serum. *Clin. Chem. Lab. Med.* 2011, 49, 1275-1281.

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Establishment of reference interval for thyroid stimulating hormone using the Bootstrap analysis and strict reference individual selection criteria from the Korean nationwide data

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Background: Serum thyroid stimulating hormone (TSH) level was influenced by several factors, including age, gender, smoking and intake of dietary iodine. We evaluated several factors specific reference interval of serum TSH levels using the Bootstrap analysis and strict reference individual selection criteria from the Korean nationwide data, a country known to be an iodine uptake excess area and **Methods**: The sixth Korean National Health and Nutrition Examination Survey (2013-2015) is a nationwide, cross-sectional survey of the Korean general population. Initially, a total of 6,905 participants aged over 10 years who underwent TSH and urinary iodine level measurements were selected. 2,582 participants who had any cases of acute or chronic disease and those taking the drug as related diseases in the health questionnaire data and 2,455 participants whose another laboratory results were abnormal were excluded. After excluding 127 statistical outlier cases, the 1,741 participants were included. TSH and urinary iodine (UI) levels were measeured by an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) and inductively coupled plasma mass spectrometry PerkinElmer, Waltham, MA), respectively. Analyse-it v 4.80 was used for Bootstrap calculation of reference interval. **Results:** The reference interval of TSH was 0.568-4.916 mIU/mL. Factor specific TSH reference interval is shown in Table 1. With the increase of UI, higher reference limit of TSH tended to increase. **Conclusions:** Strict criteria for healthy population is essential for establishing reference intervals and accurate assessment of thyroid function. We used the nationwide survey and exclusion criteria for selection of healthy to demonstrate Korean TSH reference interval. Nutritional iodine status might need to be more useful to establishing TSH reference intervals of populations in iodine -replete areas.

Table 1. Reference Interval of Serum TSH According to Gender, Age, Body Mass Index, Smoking Status and

Population			N	Reference interval of serum TSH (mIU/L)	
				Lower limit (90% CI)	Higher limit (9% CI)
Total			1,741	0.568 (0.480-0.630)	4.916 (4.764-5.0690)
	Gender	Male	1,021	0.578 (0.500-0.670)	4.981 (4.794-5.110)
		Femal	720	0.534 (0.431-0.680)	4.821 (4.619-5.050)
	Age	10-19	615	0.688 (0.570-0.800)	5.121 (4.970-5.294)
		20-29	342	0.582 (0.409-0.728)	4.883 (4.580-5.160)
		30-39	315	0.532 (0.321-0.730)	4.635 (4.431-4.940)
		40-49	225	0.621 (0.316-0.776)	4.485 (4.177-4.920)
		50-59	157	0.214 (0.010-0.484)	4.704 (4.330-5.210)
		≥ 60	87	0.362 (0.110-0.566)	4.872 (4.158-5.500)
	Body Mass Index	<18.5	208	0.584 (0.120-0.888)	5.205 (4.910-5.400)
		18.5-24.9	1,129	0.563 (0.480-0.680)	4.951 (4.780-5.090)
		25-29.9	299	0.480 (0.310-0.610)	4.670 (4.355-4.990)
		≥ 30	105	0.620 (0.260-0.850)	4.428 (4.111-4.590)
	Current smoking	No	1,467	0.576 (0.480-0.658)	5.006 (4.855-5.110)
		Yes	274	0.507 (0.425-0.627)	4.012 (3.920-4.180)
	Urine iodine (μg/L)	< 208.8	434	0.557 (0.409-0.684)	4.526 (4.363-4.671)
		208.8-364.0	436	0.751 (0.619-0.830)	4.981 (4.599-5.224)
		364.1-837.1	435	0.512 (0.377-0.673)	5.001 (4.801-5.272)
		≥ 837.2	436	0.447 (0.300-0.539)	5.038 (4.741-5.132)

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New genetic variants of hypophosphatasia. Retrospective study of hypophosphatasia in Granada, Spain

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Background: Hypophosphatasia (HPP) is a rare, serious and potentially mortal genetic disease caused by one or several mutations in the gene coding for the alkaline phosphatase without tissue specificity (TNSALP). A low serum total alkaline phosphatase (ALP) level, associated with clinical and radiographic findings, is the hallmark for the diagnosis of HPP. The childhood form of the disease is characterized by rickets-related deformities of the skeleton, craniosynostosis, delays in walking, short stature, fractures and bone pain, and early teeth loss. The diagnosis of adult forms of HPP is a challenge; patients typically present in middle age with recurrent poorly healing metatarsal stress fractures or atypical diaphyseal subtrochanteric femoral fracture; they have also an increased risk of chondrocalcinosis, enthesopathies with ossifications, and even calcific periarthritis. **Objective:** To assess the recognition of persistent low ALP levels in a tertiary care hospital in Granada to identify the patients affected with HPP, to provide an appropriate management avoiding potentially harmful drugs as antiresortive treatments. **Methods:** Between 1st of January and 31st of December 2016, 78590 patients had ALP assessment in the Biochemistry Department of our hospital. The database was divided into adult population and pediatric population. Ninety-eight patients (66 adults and 32 children) had several serum ALP values persistently below the reference interval (30 IU/l for adults, 100 IU/l for children 0-12 years old and 50 IU/l for children 13-19 years old). Through summary discharges consulting, 22 patients were discarded because of potential causes of secondary HPP. Twenty-four potential HPP patients were contacted to fulfill a questionnaire about clinical manifestations potentially related to HPP. We sampled with EDTA total blood these patients for the determination of pyridoxal-5'-phosphate (PLP) that was determined by high-performance liquid chromatography (HPLC), as well as amplification and subsequent sequencing by PCR of the coding regions and exon-intron junctions of the ALPL gene, using as reference the truncated NM_000478.4. The variants found were validated with a PCR repeat and the altered sequences. This project was approved by Ethical Committee from Andalucía Biomedical research. **Results:** 0.12% of patients who had routinaire biochemical tests along 2016 in Granada, had persistently low value of ALP. Twenty-four patients were contacted. Among them, 10 patients had fractures; 4 had symptomatic chondrocalcinosis and 4 had dental abnormalities. In all of them, ALP and PLP levels were determined and genetic analysis was performed. Nine adults and 2 children presented decreased ALP and increased PLP levels compared to the reference interval. From these 11 patients, 7 adult patients presented TNSALP mutations, 4 of them corresponded to new pathogenic variants not described previously. **Conclusions:** The study supports the effectiveness of performing screening with persistent low levels of ALP to detect cases of PPH. Our study shows that there are several omitted diagnostics of HPP in a tertiary care hospital. From 78590 patients analyzed, 7 of them presented TNSALP mutation obtaining 4 new genetic variants. These data indicate 9/100000 of HPP prevalence in Granada.

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Spironolactone metabolite causes falsely increased progesterone in the Abbott Architect Immunoassay

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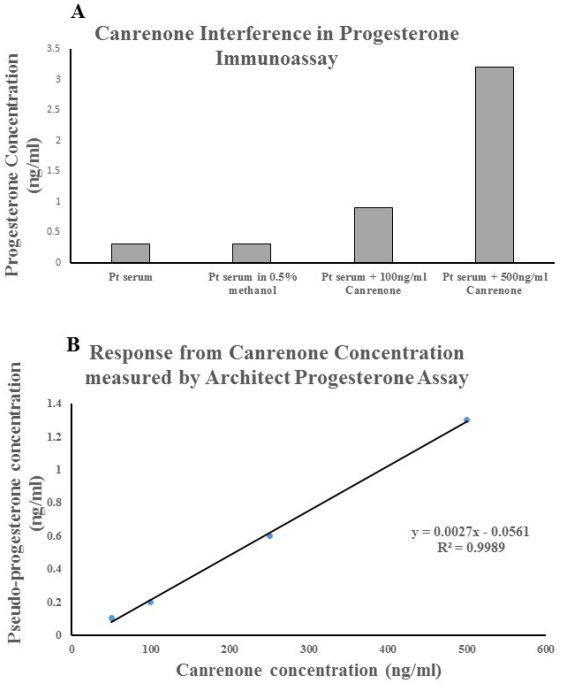
Background: Clinical laboratory determination of progesterone is a critical component for evaluating women for infertility. Primary hirsutism and anovulation are typically treated with spironolactone, a potent androgen-receptor antagonist. We identified discrepant serum progesterone measurements in patients who were prescribed spironolactone when measured on the Abbott Architect compared to the Siemens Immulite. Although spironolactone and its clinically active metabolite (canrenone) are structurally similar to progesterone, no current studies have elucidated the interference of this medication or its active metabolites in immunoassay-based progesterone measurements. **Method:** Progesterone concentrations were compared on two different immu-

noassay analyzer platforms, Abbott Architect i2000 and Siemens Immulite 2000 XPi. To investigate spironolactone and canrenone as possible interferants, we designed interference studies with commercially available material as recommended by the CLSI EP7-A2 clinical laboratory guideline. Sprinolactone or canrenone (at physiologic concentrations) was spiked in serum and analyzed on the Architect. **Results:** Patients on spironolactone showed increased progesterone concentrations (4 - 7 relative fold change) on the Architect system compared with concentrations obtained using the Immulite. Additional spiking studies with the parent drug, spironolactone, showed no interference. However, spiking canrenone into serum, at concentrations of 100 ng/mL and 500 ng/mL respectively, resulted in a 2- to 10-fold increase in progesterone when measured on the Architect (Fig. A). To further investigate the cross-reactivity of canrenone in the progesterone assay, increasing concentrations of canrenone was added into the assay-specific diluent. This produced a linear response with a positive slope on the Architect (Fig. B). **Conclusion:** The data shows a linear relationship between the concentration of canrenone and progesterone measured by the Architect assay. We report, for the first time that canrenone, a metabolite of spironolactone interferes with the Architect progesterone assay and falsely increases the results.



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Utility of thyroid function reflexive testing in inpatients with comorbidities

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Background: The American Thyroid Association, American Association of Clinical Endocrinology and National Academy of Clinical Biochemistry committees have all recommended a thyroid-stimulating hormone (TSH)-first algorithm for screening of thyroid function disorders. Simultaneous testing of TSH and free thyroxine (FT4) is recommended only for patients with unstable thyroid status, such as during the first 2-3 months of treatment for hypo- or hyperthyroidism, in hypothyroid patients suspected of non-compliance with levothyroxine replacement, and in acute conditions. However, in managing patients with comorbidities, many physicians still order TSH with FT4 due to concerns of sick euthyroid syndrome, or unstable thyroid hormone balance. This ordering pattern may lead to unnecessary testings. The study objective was to investigate if a thyroid function testing algorithm with TSH reflexed to FT4 could be applied to patients with comorbidities in inpatient settings. **Methods**: We randomly selected 100 pairs of TSH/FT4 test results at Texas Children's Hospital in-patient laboratory from September to December 2017. FT4 was measured on Vitros 5600 (Ortho Diagnostics), and TSH was measured on Architect i1000SR (Abbott Diagnostics). Patients' medical records were reviewed by clinical chemists and were grouped based on screening or monitoring purposes of the test orders, and based on physician specialty. The screening scenario was defined as no previously identified thyroid or pituitary disorders in the patient, and monitoring scenario was defined as known histories of thyroid or pituitary disorders, or histories of abnormal thyroid test results in patients. **Results**: Out of the 100 paired TSH/FT4 orders, there were 34 orders for monitoring purpose, which included 9 patients on thyroid hormone replacement or TSH inhibition therapy. The rest of the 66 orders were for screening thyroid function purposes. Among the 66 screening orders, 16 were from patients presenting to the emergency department, 9 from endocrinologists, 8 from critical care units, 8 from hematology-oncologists, and 3 as part of pre-operative testing. More importantly, of the 100 TSH/FT4 pairs, 67 showed TSH and FT4 levels both within the reference interval (RI) and 29 had an abnormal TSH and only 5 also had an abnormal FT4. Only 4 pairs had a normal TSH and abnormal FT4 and would not have been captured in a TSH first reflexive algorithm. Of these 4 pairs, two fell into the monitoring group where 1 patient was a pregnant woman with diagnosed Graves' disease, and the other patient was diagnosed with panhypopituitarism. The other two pairs of TSH/FT4 screening orders were from a patient with microcephaly, hypothermia and a patient with septicemia, both in critically ill state. **Conclusion**: This study of 100 paired in-patient TSH/FT4 orders revealed that an algorithm of TSH reflexed to FT4 would be able to capture the majority (96%) of thyroid function disorders in patients with multiple comorbidities. This would result in effective utilization of thyroid function tests and decrease test costs for the patient. Simultaneous measurement of TSH and FT4 should not be encouraged for thyroid function disorder screening purposes, except in patients in acute care setting, or when there is indication of unstable thyroid status.

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Evaluation of plasma adropin levels and cardiometabolic risk indices in type 2 diabetic patients in south west nigeria

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Introduction: Diabetes mellitus is a group of metabolic disorders characterised by dyslipidaemia and hyperglycaemia due to reduced insulin secretion or insulin resistance. Adropin as a peptide hormone has been reported to promote insulin sensitivity and secretion. **Objective:** This study was designed to evaluate plasma adropin levels in type 2 diabetic patients in South-West, Nigeria **Research Design and Methodology:** A total of 130 patients (37 males, 93 females) with age range of 42-70 years diagnosed as type 2 diabetes mellitus, were recruited for this study. Forty three apparently healthy volunteers (16 males, 27 females) with age range of 30-55 years, were included as controls. Adropin hormone level was determined by ELISA technique. Fasting plasma glucose, lipids, lipoproteins and anthropometric indices were determined using standard procedures. **Results:** The results showed significant increases in waist circumference, hip circumference, pulse rate ($p = 0.000$), waist to hip ratio ($p = 0.006$) and systolic blood pressure ($p = 0.05$) when compared with the control values. Conversely, there were significant decreases in height ($p = 0.005$) and diastolic blood pressure ($p = 0.004$) when compared with control values. There were also significant increases in fasting plasma glucose, total cholesterol, triglyceride, low density lipoprotein cholesterol/high density lipoprotein cholesterol, total cholesterol/high density lipoprotein cholesterol ratio ($p = 0.000$) when compared with the control values; while there was significant decrease in high density lipoprotein cholesterol when compared with the control values. There was a remarkable significant decrease in adropin ($p = 0.000$) when compared with the control values. **Conclusion:** The results from the present study provide evidence that decreased levels of adropin, high density lipoprotein cholesterol coupled with increased levels of fasting plasma glucose, total cholesterol, triglyceride, low density lipoprotein cholesterol/high density lipoprotein cholesterol, total cholesterol/high density lipoprotein cholesterol ratio are the main biochemical changes associated with type 2 diabetes mellitus patients in Nigeria, and therefore, they could be at increased risk of cardiovascular disease.

Keywords: Adropin, diabetes mellitus, dyslipidaemia, hyperglycaemia

Tuesday, July 31, 2018

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

Factors Affecting Test Results

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Serial sample dilutions as an initial and rapid means of evaluation for biotin interference in immunoassays: an example using the Roche high-sensitivity troponin T assay

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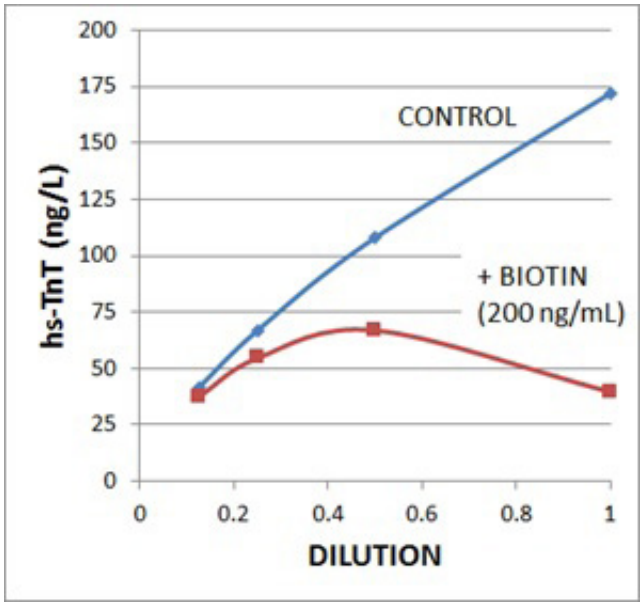
BACKGROUND: Many immunoassays utilizing biotin-streptavadin interaction are subject to interference in patients having overtly high biotin subsequent to self-administration or prescription. Potential for biotin interference with troponin measurements to mislead evaluations of acute coronary syndrome is of particular concern. Whereas stripping of biotin from patient samples using pretreatment with streptavadin is one certain way of demonstrating interference, we examined the premise that dilution series of affected patient samples was likely to be non-linear, which would be useful as an initial rapid means of assessment for biotin interference. **METHODS**: Biotin was purchased from Sigma to produce a stock solution (1000 ng/uL) used for spiking of patient samples. Pooled plasma samples (P) with elevated high-sensitivity troponin T (TnT, Roche assay) were produced by mixing of patient samples. Dilution series using Roche universal diluent were compared between control P samples and biotin-spiked P samples. A successive 2-fold dilution series produced TnT concentrations of 1, 0.5, 0.25 and 0.125 relative to P. **RESULTS**: In dilution series experiments, a wide range of initial conditions for TnT and biotin concentrations showed results for biotin-spiked samples that were highly non-linear and distinct from control series. A representative example of results for a dilution series experiment is shown in Figure, for initial conditions of TnT = 174 ng/L, spiked biotin = 200 ng/mL. Slopes between any successive points for spiked samples were distinguishable from all parts of the control curve, especially including the first sequence in which dilution produced an increase in measured TnT. **CONCLUSIONS**: Non-linearity of dilution series results for TnT can be an indication of whether biotin interference is operative in TnT measurements. As the TnT assay is of 9 min duration, dilutions may be useful as a rapid initial means of evaluation for interference relative to a procedure involving biotin-stripping sample pre-treatment.



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Reagents and Methods for Clearing Interfering Biotin and Lipemia

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Background: The presence of lipemia (elevated triglyceride-rich lipoproteins, TRL) and biotin in clinical specimens is problematic. Lipemia is a common cause of interference with routine chemistry tests. LipoClear® is available to clear lipemic samples; however, there may be loss of proteins and other analytes. We feel that an immunological approach will provide greater specificity in removing triglyceride-rich lipoproteins without affecting non-lipid analytes. Recently, the FDA has received several reports of adverse events due to biotin interference and released a communication in November 2017 warning of the potential dangers. Given these challenges, we have developed reagents and procedures to specifically deplete clinical samples of these interferents. Methods: Lipemia clearing reagent uses goat anti-human apo B to immunoprecipitate apo B-containing lipoproteins. Delipidated serum was spiked with TRL (INT-01T, Sun Diagnostics) at concentrations of 50, 100, 250, 500, 1000, and 1500 mg/dL triglycerides. Each sample (200 µL) was transferred into a a 0.45 µm microcentrifuge filter unit, 200 µL of reagent was added, and the samples were vortexed. The samples were incubated for five minutes at room temperature, then centrifuged at 12000 rpm for five minutes in an Eppendorf microcentrifuge. The filtrates were visually inspected for turbidity/lipemia. In the biotin clearance study, one mL of defibrinated serum (with no measurable biotin) were spiked with biotin (B0381, Sigma-Aldrich) at levels of 50, 100, 250, 500, 1000, 1250, and 2000 ng/mL. Samples were split into two aliquots: one with added 0.9% normal saline (100 µL, control), the other with 100 µL high capacity biotin binding resin. The samples were vortexed, incubated at room temperature for five minutes, and centrifuged at 12000 rpm in an Eppendorf microcentrifuge. The filtrates were assayed for biotin using an in-house biotin ELISA with a limit of detection of ~2 ng/mL. Results: Lipemia clearing treatment resulted in the complete clearance of turbidity at all triglyceride concentrations. Biotin clearing treatment demonstrated complete removal of biotin up to at least 2000 ng/ml. Conclusions: There is a need in the clinical laboratory community for simple and robust methods of eliminating assay interference to reduce the potential for erroneous test results. Our feasibility experiments show that lipemia and biotin clearance procedures are simple and efficient, and may be used routinely in clinical labs to increase the quality of patient test results.

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BiochemicalParameters not affected by Pneumatic Tube Delivery

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Background: Transport of clinical laboratory specimens via a pneumatic tube system (PTS) is a ubiquitous feature in any major hospital on a large campus. Some PTS pipelines can extend many kilometers and with travel speeds of 3 - 6m/s; specimens thus subjected to rapid movement, pressurization and even light exposure. Studies have cautioned the effects of PTS contributory pre-analytical factors to some laboratory test results. In this study, we considered our institution's PTS (Telecom, NL) on arterial blood gas (ABG) analyses and common biochemical parameters. **Methods:** Blood specimens sent via PTS for two in-house Point-of-Care-Testing (POCT) quality assurance exercises 6-months apart (EQA-1, EQA-2) were evaluated by comparison to central laboratory results. A later exercise was asked of POCT users to send paired ABG specimens to the central laboratory - one via PTS and one hand-carried. A data-logger (collecting data on speed, acceleration and deceleration, pressure, lighting and temperature) was placed in the PTS alongside the specimens to elucidate journey conditions. **Results:** 11 of 17 (EQA-1) and 16 of 20 (EQA-2) ABG specimens registered pO2 results within +/-10% of the central laboratory results, the remainder gave pO2 results in the range of +11.7 to +36.2% of the central laboratory results. All specimens recorded full successes for pH, pCO2 and ionic calcium. On repeat ABG testing, after taking extra care with pre-analytical factors; especially ensuring expulsion of air bubbles and airtight ABG syringes in addition to prompt dispatch of specimen on ice, all aberrant pO2 results were redeemed. 3 of 7-PTS and 5 of 7-hand specimens in the paired-specimen study yielded pO2 within +/-10% difference; difference between PTS and hand specimens was not statistically significant (p=0.29; Fisher's Exact test). 4 of 5 ABG specimens sent separately by hand (unpaired) had successful pO2 returns. All specimens again demonstrated acceptable comparability for pH, pCO2, ionic calcium, sodium and potassium. Datalogger records for the PTS journeys indicated the same motion, pressure, lighting and temperature parameters; inferring that the specimens encountered consistent PTS travel conditions. **Conclusion:** Our study showed that common biochemical analytes are unaffected by delivery via the PTS mode. PTS travel can exacerbate the inaccuracy of pO2 results if air bubbles have not methodi-

cally been removed, as evident by the redemption of pO2 results after care was taken to eliminate air bubbles and ensure airtight syringes. Notwithstanding, occurrence of deviant pO2 results in the hand-carried specimens imply that regardless of delivery mode, time-tested practices to expel air bubbles and ensure airtight ABG syringes, alongside attention to other important pre-analytical factors such as prompt dispatch and transportation on ice, should be universally employed to optimize pO2 results.

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Two high dose hook effect cases be found on alpha-fetoprotein assay in clinical practice measured by UniCel® DxI in 2017

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Background: Chemiluminescence immunoassay as a non-radioactive labeled immunoassay because of its short measurement time and no radioactive contamination, nowadays widely used in clinical laboratory analysis. Because immunoassay method based on antigen-antibody reactions, there is a certain risk of hook effect. Sandwich-type immunoassays limited by the high-dose hook effect, which will cause falsely low results when analytes are present at very high concentrations. Although manufacturers already done a comprehensive inspection and evaluation of commercial reagents in order to avoid the hook effect or to minimize the probability of occurrence of such events, and notify the risk to users in the instruction with reagent pack. Actually, such incidents still cannot find out or avoid just base on the results of the experiment or the state of the instrument. This study reviewed and analyzed 704 cases of quantitative AFP data from January to December 2017 exploring whether there is a risk of high dose hook effect in clinical practice.
Methods: All the patients who enrolled are monitoring for treatment after the diagnosis of hepatocellular carcinoma. AFP concentration were measured in routine clinical practice on Beckman Coulter UniCel® DxI 800 Immunoassay System (BECKMAN COULTER, USA) using two different items supplying by the immunoassay system named AFP and d-AFP. The two test items have different Analytical Measurement Range (AMR). The AFP item is range from 0ng/ml to 3000ng/ml, and the d-AFP item is range from 2550ng/ml to 303,000ng/ml, which based on the AFP item diluted using UniCel® DxI Access Immunoassay Wash Buffer II(REF:A79784, BECKMAN COULTER, USA) by 101 fold. All sample data greater than 303,000ng/ml will manually dilute by Wash Buffer II(REF:A16793, BECKMAN COULTER, USA) 10-fold and measured by the d-AFP item. We collected 704 cases on both AFP and d-AFP two items test data from January to December 2017 for retrospective analysis.
Results: 704 cases are reviewed included a range of concentrations ranging from 0.97ng/ml to 1734592ng/ml with a mean of 72692.77ng/ml, a median of 12805ng/ml and an interquartile range (IQR) of 52483.75ng/ml. All case data are ordered in ascending sequences from 1-704. The number of samples less than 3000ng/ml is 87 cases (12.4%), the number of samples from 3000ng/ml to 303,000ng/ml is 575 (81.7%) and the number of samples over 303,000ng/ml is 42(5.9%). No hook effect exist below 303,000ng/ml. Therefore, we focus on this part of the cases, which is over 303,000ng/ml. 39 cases in 303,000 to 1,000,000ng/ml range and no hook effect existed. Two of three cases over 1,000,000ng/ml got hook effect. Data 703 resulted in AFP item is 2672.77ng/ml and d-AFP is greater than 303,000ng/ml. After 10-fold manual dilution, d-AFP analysis data is 15,16050ng/ml. Data 704 resulted in AFP item is 1295.757ng/ml and d-AFP is greater than 303,000ng/ml. After 10-fold manual dilution, d-AFP analysis data is 1734592ng/ml.
Conclusion: For patients who following up the therapy diagnosed with hepatocellular carcinoma, especially those with past results over 500,000ng/ml. Two items both AFP and d-AFP are high recommend in order to avoid erroneous results due to high-dose hook effect which will ultimately misleading patient's assessment of treatment.

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Commutability of reference materials for HbA1c measurements: A cross-platform study

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Background:
The significance of HbA_{1c} as an important indicator for assessment of glycemic control and prediction of risk for diabetes-associated complications has been widely recognized. Comparability of results over time and across assays is essential for ensuring appropriate diagnosis and management of diseases. For this to be realized, it is necessary to have the higher order methods and certified reference materials for establishing traceability of the testing results. To select the suitable reference material formats that are commutable for routine HbA_{1c} methods based on a variety of analytical principles, a cross-platform commutability assessment study for HbA_{1c} measurements was carried out in accordance with Clinical and Laboratory Standards Institute (CLSI) Guidelines EP30-A.
Methods:
A specialized central laboratory was established for HbA_{1c} commutability validation. 50 of fresh EDTA whole blood samples with the HbA_{1c} concentration range of 0 to 130 mmol/mol were used as the individual native clinical sample. Fresh patient samples, pooled frozen blood samples, pooled and individual human hemolysate buffer with different HbA_{1c} levels were measured with 13 different analytical systems simultaneously at the central laboratory. A modified IFCC reference measurement procedure based on LC-MS/MS was served as the comparative method. The analysis was based on linear regression using ordinary least squares and the calculation of the 95% prediction intervals (PIs). Measurement results of the processed materials were compared with the limits of the PIs, and materials with results within the PIs were considered commutable. For the estimation of commutability-related biases for non-commutable materials, relative differences of measured values from predicted values were calculated.
Results:
Pooled frozen whole blood samples were commutable for almost all method comparisons, except for the Arkray HA-8180. It is evident that pooled and individual human hemolysate buffer were commutable for all analytical systems based on ion exchange HPLC (Arkray HA-8180, Bio-Rad D-10, Bio-Rad Varient II Turbo, Mindray H50, Runda MQ-2000PT, Runda MQ-6000 and Tosoh G8) and the methods based on Boronate affinity (Premier Hb 9210) as well as Capillary electrophoresis (Sebia Capillarys 2 Flex Piercing). On the other hand, it showed considerable lack of commutability for most routine methods with biochemical analyzer (Hitachi 7180 automatic biochemical analyzer combined with Mindray, Sekisui and Maccura), except Roche Cobas 501. It appears that commutability of reference material were probably correlative with concentrations.
Conclusions:
The frozen whole blood sample was commutable for 12 of 13 method comparisons, only one comparison more than expected given the 95% PI acceptance limit. The reference materials for glycated hemoglobin in human hemolysate buffer were validated commutable for the routine measurement procedure based on chromatographic method including ion exchange HPLC, boronate affinity and capillary electrophoresis. However, for methods based on enzymatic assay or immunoassay, the commutability of the hemolysate is method-dependent and associated with the concentration. Thus, it is highly suggested to identify the commutability when these formats of reference materials are used to provide traceability and evaluating the accuracy of HbA_{1c} measurements based on different methodologies.

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Pneumatic Tube System Validation for Sample Hemolysis using a Smart Phone Application at Tertiary Medical Care Institute

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Background: Pneumatic tube system (PTS) is used for speed blood transportation inside the main hospitals. It was thought that the increase in parameters of acceleration and distance of the PTS is directly associated with the increase in blood hemolysis. Therefore, in this study a smartphone application was used for monitoring such parameters to validate PTS for hemolysis at tertiary medical care institute of King Abdulaziz Medical City Hospital-Jeddah, Saudi Arabia.
Methods: A smartphone was sent (in triplicate) through the PTS from 10 different wards to the main laboratory reception. Five wards with high rates of hemolysis and five wards with low rates. Rate of hemolysis were obtained retrospectively from the number of hemolyzed samples received between Jan 2015 to Dec 2016. While the smartphone application is on the linear accelerator sensor records the three dimensions of acceleration (parameters X, Y and Z (m/s^2)) against the variable of time in second.
Results: The minimum and maximum values for variables of acceleration in wards with high rates of hemolysis [X (0.13 to 0.46 m/s^2), Y(-0.10 to 0.08 m/s^2) and Z (-0.23 to 0.53 m/s^2)] were not changed significantly from the same variables in wards with low rates of hemolysis [X (-0.09 to 0.26 m/s^2), Y (-0.11 to 0.24 m/s^2), Z (0.02 to 0.48 m/s^2)]; p values for X= 0.50, Y= 0.34 and Z= 0.08 respectively. There was no significant correlation between the time required for transportation and the number of hemolyzed samples ($r^2 = 0.19$, $p = 0.32$).
Conclusions: It seems that the reason for sample hemolysis at the institute of King Abdulaziz Medical City-Jeddah is not due to sample acceleration in the PTS but it could be due to other preanalytical or analytical factors.

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Influence of a Chilean standardized breakfast on routine hematological tests

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Background: Among the requisites of the preanalytical phase, fasting time is an issue of relevant concern. Yet, fasting status is not always investigated by laboratory staff before blood sampling for hematology assays. In this study, we assessed whether a Chilean standardized breakfast might bias the results of routine hematological tests. **Methods:** Blood samples were collected from 10 healthy volunteers by a single, expert phlebotomist, using a 21 G straight needle (Vacumed 45203, Torreglia, Italy), directly into 3.0 mL evacuated tubes containing K_2 EDTA (Vacumed 42011, Torreglia, Italy). A first blood sample was collected between 8:00 and 8:30 a.m. after an overnight fast. Immediately after blood collection, the volunteers consumed a breakfast containing a standardized amount of carbohydrates, proteins, and lipids. Subsequently blood samples were collected 1, 2 and 4 hours afterwards. Each phase of sample collection was carefully standardized, including the use of needles and evacuated tubes from the same lot. Samples were assayed on the same Sysmex XE2100D, Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan). Differences between samples were assessed by Wilcoxon ranked-pairs test. The level of statistical significance was set at $P < 0.05$. **Results:** The results of this investigation are presented as median [interquartile range] in Table 1. **Conclusion:** The significant variations observed in several hematological parameters due to breakfast consumption demonstrate that the fasting time needs to be carefully taken into account prior of performing hematological assays, particularly as regards neutrophils, lymphocyte, monocyte, red blood cell, hemoglobin and hematocrit, in order to avoid interpretive mistakes of test results, and to guarantee patient safety.

Table 1. Postprandial variation on routine hematological tests

Parameters	Basal	1h	P	2h	P	4h	P
white blood cells	6.30 [5.56 - 9.06]	6.26 [5.34 - 9.13]	0.695	6.48 [5.63 - 9.04]	0.232	6.96 [5.92 - 10.0]	0.078
neutrophils	3.39 [2.88 - 5.57]	3.81 [3.23 - 5.88]	**0.031**	4.02 [3.40 - 5.98]	**0.034**	4.08 [3.45 - 6.06]	**0.023**
lymphocytes	2.26 [1.74 - 2.68]	1.76 [1.47 - 2.20]	**0.002**	1.98 [1.64 - 2.59]	**0.044**	2.37 [1.77 - 3.09]	**0.025**
monocytes	0.44 [0.42 - 0.56]	0.37 [0.34 - 0.50]	**0.027**	0.44 [0.37 - 0.54]	0.683	0.52 [0.40 - 0.66]	**0.039**
eosinophils	0.15 [0.10 - 0.26]	0.13 [0.06 - 0.20]	0.154	0.12 [0.06 - 0.23]	0.152	0.12 [0.08 - 0.21]	0.207
basophils	0.04 [0.03 - 0.04]	0.04 [0.02 - 0.04]	0.089	0.04 [0.03 - 0.05]	0.345	0.03 [0.03 - 0.05]	0.357
red blood cells	4.76 [4.66 - 4.93]	4.70 [4.60 - 4.94]	0.202	4.64 [4.54 - 4.94]	**0.011**	4.60 [4.56 - 5.00]	**0.016**
hemoglobin	14.4 [13.7 - 15.0]	14.3 [13.9 - 15.1]	0.904	14.1 [13.6 - 14.8]	**0.034**	14.0 [13.9 - 15.1]	**0.038**
hematocrit	45.0 [43.6 - 46.9]	44.5 [43.4 - 47.2]	0.307	42.9 [42.0 - 46.9]	**0.008**	42.7 [41.6 - 46.4]	**0.007**
platelets	252 [223 - 313]	253 [220 - 322]	0.284	252 [224 - 318]	0.358	260 [217 - 329]	0.160

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Study of the effects of the execution time on the basic coagulation tests in patients with and without anticoagulant treatment

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Background: Coagulation tests are susceptible to much interference in the results: environmental factors, especially temperature, collection material, specific anticoagulant, manipulation of the collection tube as well as centrifugation, among others, play an important role on these tests. One of the great challenges of the clinical laboratories is to perform tests of coagulation in time recommended by Clinical and Laboratory Standard Institute (CLSI). The laboratories that have many units of collection, the care in the transportation to the place of realization of the tests is fundamental. In the literature there are many studies that aim at the control of interference factors for the accomplishment of these exams according to their needs. In this study, the authors needed to identify a maximum time of execution of the basic coagulation tests, keeping them at room temperature (RT) and without centrifugation until the execution time, without impact on the results. Methods We selected 100 patients from the laboratory routine divided into: 50 without treatment and 50 with treatment (one Warfarin group and another with Aspirin). After They sign free and informed consent term, , 5 tubes of venous blood were collected with sodium citrate 0.109 mol/L -3.2WV% Vacuette® from each volunteer. The samples were transported on shelves, in boxes with RT (18-25° C) and without centrifugation, from the collection unit to the laboratory where the tests would be carried out. Each sample was performed at a given time (0,4,8,16 and 24 hours). The centrifugation was performed prior to the examination only at their respective times. The tests performed for each sample were (thrombin time (TP), Prothrombin activated (PA), International Normalized Ratio (INR), activated partial thromboplastin time (APTT) determined by the coagulometer in Sysmex ® CA 1500 equipment. . ResultsWe performed statistical tests of repeated measures of ANOVA with poshoc in groups of: without medication, group with Aspirin and group with Warfarin and comparing posterior and basal times: having as dependent variables the following measures: TP, PA, INR , And independent variables such as execution times (0,4,8,16,24horas), we observed that: the results of the statistical tests showed that for all variables there is no interaction effect between the presence or absence of drugs with the waiting time.Based on the post-hoc results, TP, PA and INR measurements did not present significant differences between baseline values and times 4, 8 and 16, presenting a difference in relation to the 24-h post-test ($p < 0.006$), in RT.The APTT measures presented a significant difference with the baseline measurement at 8, 16 and 24 hours ($p < 0.001$). The same for both treatment groups. Conclusion The authors concluded that the factor that determines the differences in the 5 measures is the waiting time regardless of the use or not of medication.The study shows that the TP, PA and INR tests can wait until 24h in tube, whereas the APTT and Relay measurements only up to 8h, both at RT and without centrifugation.The groups with drug treatments presented the same behavior of the group without treatment.

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Frozen serum stored in Gel Separator primary sampling tubes: Is stability affected for testing in endocrinology and serology?

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Background: Serum separator tubes were introduced into laboratories approximately 25 years ago and since have gained widespread acceptance due to the advantage of a barrier gel that facilitates rapid separation of serum from cells. Use of these tubes makes drawing blood easier, facilitates blood clotting and rapid separation of serum, reduces centrifugation time (they withstand higher centrifugation speed), and avoids transfer of serum to new tubes, contributing to improved quality of the preanalytical phase. In clinical practice laboratories store biological samples for varying times and temperatures according to the needs. Samples are stored for short time periods when they are processed in batches, for re-measurement, or when they are referred from or to other laboratories. Samples that are stored for a longer time could be used in studies of diagnosis-related groups, assessment of reference intervals of different analytes, or for general scientific purposes. Kutasz et al have described analytes whose results are statistically within the range of uncertainty of measurement after a period of storage at a certain temperature as stable, analytes within the range of the Reference Change Value as clinically useful, and analytes outside these range as unstable. **Objective:** To assess if storage of serum stored in gel separator primary sampling tubes at -20°C affects the results and the stability of endocrinology and serology tests after three months. **Methods:** Sera from adult donors for IgG serology of Hbs, CMV, EBV (Architect i4000) and varicella (VIDAS), for endocrinology, TSH, fT4, LH, FSH, insulin, Vitamin D and estradiol (Architect i4000), GH, IGF1 and Cortisol (Immulite 2000), were assesed. The samples were measured at time 0 with (t0gel) and without (t0) gel and at time t3 (3 months) with (t3gel) and without (t3) gel. We compared different times with and without gel (t0gel vs t0 and t3gel vs t3) according to Bland and Altman methods modified by Andersen to check if the gel affects the results obtained, if the ratios were within the range of analytical variation, the gel was considered not to affect de results. The stability of the sample stored at -20°C for three months was studied comparing t3 with t0 according to the Kutasz criteria . **Results:** In the studied times the analytes had not difference between the stored with and without gel. After a 3-month period of storage at -20°C TSH, fT4, FSH, cortisol, anti HBs, CMV, EBV and varicella remained stable; ; LH, Insulin, VitD, GH and estradiol were clinically useful; and IGF1 was unstable. **Conclusions:** Storage in gel separator primary sampling tubes for three months at -20°C does not affect the quality of the sample for further testing. Effect on stability is mainly due to the freezing and thawing process rather than to the presence of the gel.

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Pneumatic Tube Delivery of Whole Blood Specimens Affects the Measurement of Lactate Dehydrogenase, Total CO2 and Anion Gap

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Background: Pneumatic tubes (P-tube) have been widely used to transport specimens in medical centres to improve turnaround times. In this study, we evaluate the impact of transporting whole blood specimens through a P-tube on the measurement of electrolytes, total CO_2, anion gap and lactate dehydrogenase (LD). **Methods:** Two whole blood specimens of equal volume were collected into lithium heparin Vacutainer® tubes from 40 ambulatory patients. The P-tube was used to transport one tube from each patient while the second tube was delivered by walking. Sodium, potassium, chloride, Total CO_2, LD and anion gap were measured using the Roche Cobas® 6000 chemistry analyzers. **Results:** The following Passing Bablok regression equations describe the relationship between the specimens sent by P-tube and the hand delivered specimens: Sodium: y = 1.0x - 1.0, R^2= 0.650; Potassium: y = 1.0x + 0, R^2 = 0.762; Chloride: y = 1.0x + 0, R^2 = 0.709; Total CO_2: y = 1.0x - 1.0, R^2 = 0.667; Anion Gap: y = 1.0x + 2.0, R^2 = 0.436; LD y = 1.07x - 1.33, R^2 = 0.679. Bland Altman analysis indicated the following mean bias and (95% confidence intervals). Sodium: mean bias: - 0.4 mmol/L (-3.0 to 2.2); Potassium mean bias: 0.01 mmol/L (-0.29 to 0.32); Chloride mean bias -0.5 mmol/L (-3.0 to 2.0); Total CO_2 mean bias -1.5 mmol/L (-3.7 to 0.7); Anion gap mean bias 1.6 mmol/L (-1.4 to 4.5); LD mean bias 17.1 U/L (-26.5 to 60.7). Paired t-tests were used to evaluate statistical differences between specimens sent through the P-tube versus walked to the laboratory: Potassium p< 0.54; Sodium p< 0.051; Chloride p<0.016 ; Total CO_2 p<0.001; Anion Gap p<0.0001; LD p <0.0001. **Conclusions:** Transport of whole blood specimens obtained from ambulatory patients through the P-tube caused statistically significant differences in Sodium, Chloride, LD, Total CO_2 and Anion gap. Further studies need to be conducted to understand the extent of clinical impact of the P-tube transport on these tests.

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Validation of CSF Gentamycin Measurement by VITROS®5600

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Background: A newborn with a ventriculo-peritoneal (VP) shunt for hydrocephalus due to a tectal glioma developed bacterial ventriculitis. CSF culture grew multi-drug resistant enterobacter aerogenes while blood culture was negative. The pediatric team decided to treat this newborn with intrathecal Gentamycin. The dosage of the intrathecal Gentamycin as well as the protocol for CSF sample collections for the trough and peak CSF Gentamycin levels, were based on the guidelines for the management of intra-cranial infections in children and adults from Oxford Radcliffe Hospitals. The objective of this study is to validate CSF Gentamycin measurement by VITROS 5600 to help pediatricians manage this newborn with frequent CSF Gentamycin monitoring. **Methods:** A linearity experiment was performed with the 100mg/L Gentamycin in normal saline obtained from our hospital pharmacy. This Gentamycin stock was mixed with pooled CSF fluid samples from other patients (zero pool) from the laboratory to make a 10mg/L high pool, which was then mixed with the zero pool CSF fluid at different ratios to create the final Gentamycin concentrations at 0 mg/L, 1 mg/L, 1.25 mg/L, 2.5 mg/L, 5 mg/L, 7.5 mg/L and 10 mg/L. Each level was tested in triplicate. Accuracy was assessed by comparing each CSF Gentamycin sample from this newborn obtained by VITROS 5600 to those measured by Siemens VISTA. Within-day precision was tested 10 times at the Gentamycin concentrations of 2 mg/L and 9 mg/L and between-day precision was tested in triplicates for 5 days at the same levels. Interference study was tested with spiking the pooled CSF samples with hemolysates at the Gentamycin concentration of 5 mg/L. **Results:** The linearity of CSF Gentamycin was perfect (R^2= 0.9991). At the Gentamycin concentrations of 2 mg/L and 9 mg/L, both the within-day and between-day precisions were less than 6%. In comparison with the CSF Gentamycin measured by VISTA, the average percentage difference of Gentamycin measurements by VITROS 5600 was less than 5%. At the Gentamycin concentrations of 5 mg/L, there was no hemolysis interferences with hemoglobin up to 5 g/L. **Conclusion:** Linearity, precision, accuracy and hemolysis interference studies all suggested the CSF gentamycin measured by VITROS 56OO was validated for clinical reporting.

A-244

Potential for erroneous results in lateral flow tests employing biotin technology

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Background: It has recently been found that in vitro immunoassay systems employing biotin capture systems can be subject to interference by exogenous biotin in serum and urine of patients taking biotin supplements; with FDA issuing a safety communication on this matter in November 2017. This investigation sought to understand whether home pregnancy tests that employ biotin technology are also affected by this phenomenon. **Methods:** Urine hCG standards (50mIU/ml) were prepared to contain 0, 10, 50, 100, 200, 300, 500 and 1000nM biotin, which were chosen to represent concentrations that may be present in urine following supplement use. Due to patent protection, few home pregnancy tests employ biotin detection, therefore product testing was limited to First Response visual home pregnancy test which uses biotinylated detection reagents. Five repetitions were conducted per biotin concentration, and the line intensity was read using a camera system. **Results:** Increasing levels of biotin were found to reduce the visual intensity of the test line as shown in the table below. A camera reading of >10 would be consistently read as a positive result, whereas camera readings below this value may be read as negative results. **Conclusion:** Lateral flow tests employing biotin technology are, like laboratory biotin-based immunoassays, subject to interference from exogenous biotin that could be present in urine following supplementation. Therefore the existing warnings regarding potential for erroneous results should also be applied to home pregnancy tests that employ biotin technology.

Line intensity for 50mIU/ml hCG lateral flow test result for different biotin levles								
Biotin (nM)	0	10	50	100	200	300	500	1000
Mean Signal	16.3	16.7	16.4	14.1	13.7	12.5	7.9	4.0
S.D.	1.8	3.4	1.1	0.8	1.5	1.4	1.0	0.6
% of control	N/A	102	101	87	84	77	49	25

A-245

Validation of Precision and Accuracy for Measurement of Selected Analytes in Non-Standard Body Fluid

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Objectives: Requests to analyze non-standard body fluids are frequently requested by clinicians to investigate and manage various diseases. However, most often these matrices are not approved uses for available FDA approved systems. Thus requiring in-house validation. This study aims to validate the precision and accuracy of several analytes measured in dialysates, peritoneal fluid, and pleural fluid on a Roche cobas 6000 system (c501and e601). **Design and Methods:** Clinical specimens stored at 4 °C for 1-7 days were collected and transferred to -80 °C for storage until the day of experiments being performed. For precision study, individual samples were pooled, aliquoted, and 3 aliquots were analyzed each day for 5 days. For accuracy, high purity chemical(s) or recombinant enzyme(s) were spiked into 5 individual body fluid samples at 5 different levels. The same amounts of analytes were also spiked into pooled heparin plasma sample. A 10% (v/v) spiking volume was used to avoid significant matrix change. Accuracy of non-standard body fluid measurement was evaluated as recovered concentration compared to the recovered concentration from pooled plasma. **Results and Conclusions:** Precision and accuracy data are shown below. The current study demonstrates the applicability of this experimental design as part of complete validation of body fluid measurement as modified lab developed tests. Previous publication validated accuracy by spiking high concentration serum, control, or calibrator into body fluids. Our design differs by spiking high purity materials to create concentrations across the reportable range. Although the materials are not traceable materials, we overcome this issue by spiking these materials into pooled plasma for comparison.

		Dialysates	Pleural				Peritoneal			
		Urea	Glucose	LDH	Amylase	Lipase	Glucose	LDH	Amylase	Lipase
Precision	Intra-day CV%	0.2-1.8%	0.7-1.2%	0.4-2.8%	0.0 - 0.9%	0.2 - 1.4%	0.0-1.8%	0.7-12.7%	0.6-2.0%	0.4-6.6%
	Inter-day CV%	2.1%	1.1%	6.2%	0.8%	2.6%	0.9%	5.5%	1.1%	3.1%
Accuracy*		92.7%	102.6%	103.3	101.0%	102.6%	101.6%	N/A	101.8%	104.8%

*Average recovery efficiency at different levles

A-246

Effect of Hemolysis and Icterus on Chemistry Tests and Association between the Amount of Interfering Substances and H-index and I-index

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Background: Interference from endogenous substances is one of the leading source of errors that clinical laboratories frequently encounter at the pre-analytical phase of testing. Automated chemistry platforms allow accurate measurement of interferences due to endogenous substances such as hemolysis and icterus utilizing semi-quantitative testing with indices. We evaluated the effect of hemolysis and icterus on chemistry assays and further assessed the association between the amount of interfering substances and ordinal values reported by the automated chemistry analyzer as H- and I- indices. **Methods:** Three normal serum pools were prepared and supplemented with six increasing concentrations of hemolysate and bilirubin. These samples were then tested for 40 chemistry analytes for hemolysis and 38 chemistry analytes for icterus interferences on a Beckman Coulter AU5800 series analyzer. Results were compared to baseline values and acceptability of results were determined based on the total allowable error limits according to CAP and CLIA guidelines. The amount of hemolysis and icterus were measured using a semi-quantitative photometric test on the same instrument using the Beckman Coulter LIH reagent system. These values were assigned by the instrument on an ordinal scale as qualitative flag levels ("N", "+", "++", "+++", "++++" and "+++++") to reflect the degree of hemolysis and icterus in a specimen. Visual detection of the hemolysis and icterus was also performed independently on each aliquot in a blinded manner by three experienced technologists. **Results:** Interference from hemolysis was detected for 20 of 40 tested analytes. Half of these twenty analytes were affected by gross hemolysis at hemoglobin concentrations of 798 mg/dL with ordinal values of "+++++" flag level. Only three analytes (aspartate aminotransferase, direct bilirubin and lactate dehydrogenase) were affected by slight hemolysis at hemoglobin concentrations of 76 mg/dL with ordinal values of "+" flag level. Aldolase was the only analyte that was affected at hemoglobin concentrations of 25 mg/dL. Interference from icterus was detected for 9 of 38 tested analytes. Three of these nine analytes were affected by gross icterus at bilirubin concentrations of 60 mg/dL with ordinal values of "+++++" flag level. Free glycerol was the only analyte that was affected by bilirubin concentrations of 3.7 mg/dL with ordinal values of "+" flag level. Visual inspection results for hemolysis showed good agreement between three technologists and were consistent with the corresponding ordinal values. Visual inspection results for icterus showed more variations between technologists and compared to ordinal values. **Conclusions:** We have demonstrated that some of the chemistry analytes were affected by hemolysis and icterus interferences. Generally, our results were consistent with manufacturer's claims. Our laboratory applied the results to determine the cut-off indices for hemolysis and icterus on tested chemistry analytes using the robust measurement of the interferent provided by the automated chemistry analyzer. The implementation of the indices allows us to effectively determine the specimen integrity and prevent erroneous test results due to hemolysis and icterus.

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Implementing Kleihauer-Betke test external quality assessment scheme (EQAS), a French experience.

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BACKGROUND: The Kleihauer-Betke test (KBT) is a laboratory examination used to quantify foetomaternal hemorrhage. Although this method has proved to be useful clinically, this test is often criticized. It is a manual test with a high level of variability, difficult to standardize and requiring technical expertise. Even if the flow cytometry is used to replace the KBT, it is not widely used, unsuitable for emergency and displays limitations such as F-cells interferences. **METHODS:** Taking into account the mandatory accreditation for French Laboratories the need for an external quality assessment scheme (EQAS) raised. The CNRHP and ASQUALAB implemented an EQAS including a stained smear, a whole blood sample (a calibrated mix of fetal and maternal cells with a target value) and a clinical case study. Five surveys were conducted since 2015 gathering increasing number of participants from 57 to 146 laboratories in 2018. **RESULTS:** The interlaboratory variability ranges from 25% to 30%. The average of the laboratories is higher than the target value, mostly probably due to underestimation of adult erythrocytes count. **CONCLUSION:**This evaluation demonstrates the difficulties to standardize the KBT and the need for EQAS for competency improvement.

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The Effect of 37°C Temperature on the Stability of Routine Chemistry Analytes in Serum

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Introduction: Thousands of blood samples are transported daily by couriers from outpatient providers' offices to the Automated Chemistry laboratory. In the summer, samples stored in lock-boxes can be subjected to prolonged elevated temperatures prior to courier pick up, potentially affecting test results (highest recorded lock box temperature = 31° C). We hypothesize that such elevated temperatures may be detrimental to the stability of various chemistry analytes (particularly enzymes) in serum samples. **Study Design:** Ten remnant serum pools were prepared (no separator gel present, 24 to 72 hours old) – one normal pool and nine others representing a variety of abnormal clinical states (elevations of: creatinine, alkaline phosphatase, ALT/AST, creatine kinase (CK), PSA, and TSH). Each pool was divided into 4 aliquots. Four gold-top Vacutainer® tubes were also obtained from each of fourteen healthy volunteers (12 non-pregnant females & 2 males); these were processed immediately after collection and remained capped until testing was performed. One aliquot from each pool and each volunteer represented time zero (baseline). The remaining tubes were incubated at 37°C for 8, 12 and 24 hours, respectively, prior to testing. Pooled samples were also capped during the incubation periods. Test results were obtained for sodium, potassium, chloride, CO_2, BUN, creatinine, calcium, phosphate, magnesium, total protein, albumin, CK, GGT, amylase, lipase, total and direct bilirubin, total & HDL cholesterol, triglycerides, alkaline phosphatase, CRP, ALT, AST, free T4, TSH, folate, PSA, and vitamin B12.The Siemens Advia® 1800 and Siemens Centaur® XP chemistry analyzers were used for testing. Differences (residual and % residual) between zero time and 37°C storage times were calculated. Total Analytical Error (TAE) was employed to determine significant differences. **Results:** Of the 31 tests performed on the pooled remnants, significant decreases were noted after 8 hours for CK (average decrease at 8 hours = 55.4%, TAE 30%) and ALT (average decrease at 8 hours = 23.51%, TAE 20%) and after 12 hours for lipase (average decrease at 12 hours = 32.45%, TAE 20%), calcium (average decrease at 12 hours = 10.40%, TAE 6%), & phosphorus (average decrease at 12 hours = 13.60%, TAE 11%). Of the 31 tests performed on 14 volunteer samples (separator gel left in place in the tube), no appreciable pattern or clinically significant increases or decreases of results were identified. **Conclusion:** There was a significant decrease in CK and ALT activity when pooled serum samples were subjected to 37°C temperature for as short a period of time as 8 hours; lipase, calcium and phosphorus results were affected after 12 hours. However, no particular pattern of change was detected in the volunteer samples even after 24 hours at 37°C. The difference in results between pools and volunteer samples may in part be due to the pools already being 24-72 hours old. The effect of disease states (or sample exposure to air of the pools prior to preparation) could also have contributed to the differences encountered, although the normal pool also yielded decreases in CK and ALT.

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Evaluation of the contamination index in the urine culture after urogenital region hygiene with moist toilet tissue

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Background: Culture of a urine sample is one of the most requested exams in the microbiology laboratory. Contamination of the urine culture can lead to false positive results and, inadequate collection techniques of urine samples are considered the main reason for contamination. The urine culture contamination index is an important indicator of the quality of the laboratory's pre-analytical phase. The contamination of the urine culture can be reduced by appropriate collection, transport and storage of the urine sample. The outcome of urine culture to provide relevant clinical information depends on careful sample collection, including performing hygiene of the urogenital region. Genital hygiene with mild soap and rinse water is traditionally recommended, alternatively, the use of moist tissues in antisepsis of the urogenital region is recommended. The aim of this study is to evaluate the contamination index of urine culture with the use of moist tissues in genital hygiene. **Methods:** : We evaluated 100 urine culture of patients seen at the outpatient clinic of the University Hospital, where urogenital hygiene was performed with a moist

tissue. All collections were performed after verbal guidance and the distribution of an illustrated orientation for urogenital hygiene with two 14 x 17 cm moist wipes containing Propylene Glycol, Methylparaben, Tetrasodium EDTA, Lactic Acid, Disodium-Cocoamphodiacetate. After the cleaning procedure, mid-stream urine samples were collected in sterile, wide mouthed bottle and stored in a refrigerator until transportation and delivery in the microbiology laboratory. Strips calibrated to deliver 0.001mLl of urine on CLED (cystine lactose electrolyte deficient) agar were used. All plates were incubated at 37°C and read at 24 and 48 hours.The criteria for the definition of contaminated urine culture were: 1) count over 100,000 CFU / mL with three or more different types of colony. 2) count less than 100,000 CFU / mL with two or more different types of non-uropathogenic bacteria. The frequency of positive samples and contaminations in samples of men and women was evaluated. **Results:** Of the total number of urine culture analyzed, 64% were female and 36% male. 87% of the urine culture were negative, 11% positive and 2% considered contaminated. The contaminated urine samples were all from female patients and 82% of the positive cultures were from females. **Conclusion:** The use of moist tissues in genital hygiene seems to be adequate at keeping contamination indexes within the targets set by the analytical quality programs of clinical laboratories.

A-250

The prevalence of Biotin in samples submitted for laboratory testing. Assessment of risk for interference.

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Background:
Biotin interference in streptavidin-based immunoassays can cause incorrect laboratory results leading to erroneous results and to possible inappropriate patient management. Of 374 methods available on the 8 most popular immunoassay analyzers in the United States, 221 instruments have biotin-based immunoassays and 82 of which had manufacturer-reported interference thresholds of circulating biotin at less than 51 ng/mL. Biotin levels greater than 20 ng/mL are known to exhibit interference in troponin T (TnT), 25 ng/mL in thyroid stimulating hormone (TSH) assays, and 30 ng/mL in prostate specific antigen (PSA) assays when using our Cobas® immunoassay analyzer (Roche Diagnostics, IN, USA). The recent increase in reports of biotin interference in clinical chemistry testing has been attributed to increased purchase of biotin supplements by the public and to the high-dose biotin therapy in patients with multiple sclerosis. The aim of this study was to examine the risk for biotin interference among our patient population.
Methods:
Forty-four serum and plasma leftover samples were collected following completion of TnT (14 samples), TSH (14 samples), PSA (16 samples). Aliquots were stored frozen at -20 °C until analysis. Biotin concentrations in these samples were measured using an ALPCO Elisa kit (Salem, NH) according to the manufacture's protocol. Samples with biotin levels of 20 ng/mL or greater were considered as "high risk samples" (HRS).
Results:
The overall concentrations of biotin in the study patients' samples ranged from 0.17 ng/mL to 7.73 ng/mL (median 1.19 ng/mL). The mean and (range) biotin concentrations in TnT, TSH, and PSA sample aliquots was 1.25 ng/mL (0.19 – 3.52 ng/mL), 1.00 ng/mL (0.22 – 2.51 ng/mL), and 1.32 ng/mL (0.17 – 7.73 ng/mL) respectively. Of the 44 specimens tested, none were considered HRS as their biotin levels were less than 20 ng/mL.
Conclusion:
Using representative samples with requests for TnT, TSH and PSA (known to be most affected by circulating biotin levels), the risk for interference by biotin among this population was considered minimal. However, educating clinicians and laboratory users of the potential of biotin interference is always recommended.

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Social and Legal Implications of Urine Drug Screen Analysis in the Neonate: A Case of Suspected Specimen Mishandling

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Background: Drug screening in the newborn population comes with a unique set of analytic, therapeutic and legal caveats that make interpretation of results challenging. Additionally, because universal drug screening of newborns is impractical and is not recommended by the American Academy of Pediatrics, it is imperative that institutions have policies and procedures that clearly define criteria for screening infants suspected of *in utero* drug exposure. The study presented describes two high risk infants in which a urine drug screen (UDS) was ordered and results were inconsistent with the history provided by the mothers. Because of the recognized prevalence of specimen mislabeling, the unexpected results were initially attributed to a pre-analytic error rather than inaccurate patient history. However, alternative methods of specimen identification were employed and confirmed the identity of the specimens in question. **Methods**: The UDS was performed on the Roche c501 analyzer utilizing an immunoassay based on the kinetic interaction of microparticles in a solution (KIMS)**.** Evaluation of meconium was achieved by LC/MS/MS**.** Genotype analysis of DNA from cells isolated from urine specimens used for drug testing was accomplished with the PowerPlex® 16 HS System from Promega. This assay allows for the co-amplification and three-color detection of sixteen loci (fifteen short tandem repeat (STR) autosomal loci and the amelogenin locus for gender determination). **Results:** UDS analysis was inconsistent with clinical history as provided by the mothers. Specifically, Infant A, whose mother denied illicit drug use during pregnancy, screened positive for the presence of cocaine while her mother's urine was negative. In contrast, Infant B, whose mother admitted to poly-drug use during pregnancy was positive for methadone only, while his mother's urine screened positive for cocaine, benzodiazepine, opiates, and THC. Given the discordance of these results, hospital staff was concerned that the specimens had been mishandled. However, genotype analysis of cells isolated from the urine specimens of infant A and her mother confirmed a genetic relationship as 16/16 STR genetic markers matched. **Conclusion:** In the case presented, it was suspected that specimen mishandling was the most likely reason to explain results inconsistent with patient history. While strict adherence to established policies and procedures is designed to prevent mistakes, pre-analytic error is often suspected in cases where laboratory findings are not reflective of clinical presentation. This case illustrates how supplemental genetic analysis can be used to confirm specimen identity in cases where specimen mishandling is suspected, especially when results have serious clinical or legal ramifications.

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Efficacy of Various Estimated Creatinine Clearance Methods in Estimating Glomerular Filtration Rate in Indians

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Background: The aim of this study was to compare the efficacy of GFR derived from various estimated creatinine clearance methods like Jelliffe, Cockcroft and Gault, and 4MDRD equations as compared to measured glomerular filtration rate (GFR) with in Indians. **Methods:** : We enrolled 80 patients in the study. GFR was determined by technetium-99m diethyl triamine penta-acetic acid (Tc99mDTPA) clearance . Height, body weight and serum creatinine were measured, and GFR and creatinine clearance (CrCl) estimates calculated by various equations. Spearemans correlation was used to assess relationships between measured GFR (Tc99mDTPA clearance) and estimated clearances using the three formulae. Difference between the measured GFR and estimated clearances compared with measured GFR were examined to determine whether prediction error was independent from measurement magnitude. Analyses of differences were used to determine bias and precision. Bias was assessed by mean percentage error (MPE), calculated as the percentage difference between the estimated clearances for each formula and measured GFR. A positive bias indicates overestimation of GFR, and a negative bias indicates underestimation. Relationships were also assessed by gender and varying levels of renal function: GFR <60 ml / min, and GFR >60 ml/ min. **Results:** The mean measured GFR was 77.2 ml / min (range 17 to 152 ml / min). The mean bias (mean percentage error) was -4.9, -10.3 and -1.57% respectively for the, Jelliffe , Cockcroft and Gault, and 4MDRD formulas, respectively. The 4 MDRD formula slightly overestimates the GFR in patients having GFR less than 60ml/ min, where as,it underestimates for GFR more than 60ml / min.. **Conclusion:** In Indians 4 MDRD equation of estimated creatinine clearance seems to be most efficient in estimating GFR . :

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Changes in Biochemical Indices after Plateletpheresis in Male Donors

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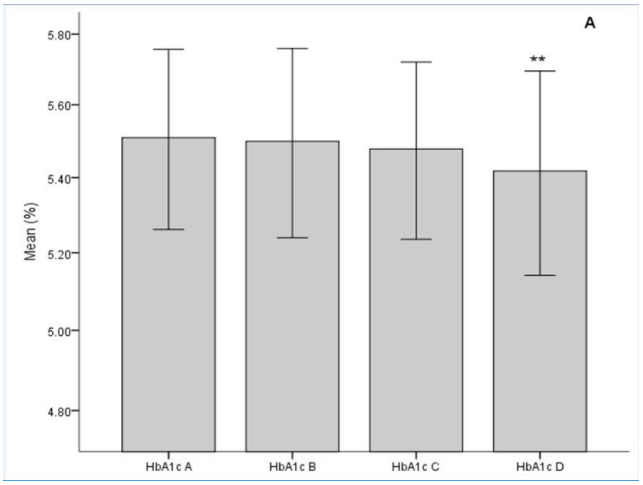
Background: There is relatively little information about endogenous biochemical changes in a response to plateletpheresis in healthy donors. We aimed to investigate the changes in different biochemical indices including glycemic status, insulin resistance, iron status, lipid profile and inflammatory markers after plateletpheresis in healthy male donors with normal glycemic status.
Methods: In this study we enrolled 15 healthy male donors. The glycemic status in all donors was assessed using an oral glucose tolerance test pre- and post-plateletpheresis at different time intervals (1, 8 and 22 days). Different biochemical indices including glucose, HbA1c, insulin, lipids, uric acid, transferrin, ferritin, C-reactive protein and insulin resistance were measured. Repeated ANOVA was utilized for the purpose of statistical comparison of means between different days.
Results: Fasting glucose, transferrin, cholesterol, triglycerides, HDL-C, and LDL-C were significantly altered (-3.9%, $p<0.05$; -2.7%, $p<0.05$; -3.9%, $p<0.05$; 23.9%, $p<0.05$; -5.5%, $p<0.01$; and -9.2%, $p<0.05$ respectively) at day 1 following plateletpheresis. There was a gradual reduction in HbA1c (Fig. A) and ferritin levels during the time-course of the study, and by day 22, both were significantly lower (-2.0%, $p<0.01$; -18.1%, $p<0.05$ respectively) when compared to the pre-plateletpheresis levels.
Conclusion: After plateletpheresis, several biochemical indices may change significantly in healthy male donors. The changes were particularly evident 1 and 22 days post-donation. The potential effects of plateletpheresis need to be considered when interpreting biochemical tests.



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Effect of open containers on stability of common plasma chemistries measured on total automation lines

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Background:
Understanding pre-analytical variables in automation-line testing of patient samples is crucial for determining whether test results are accurate. The present study was designed to determine how plasma samples aliquoted and placed in sample cups for automated line testing compare to samples that are collected in the original tubes. The samples were tested for common analytes in the comprehensive metabolic panel.
Method:
Samples were drawn from 5 apparently healthy volunteers (ages 30-62 years) in lithium heparin tubes. The samples were centrifuged per laboratory protocol. The samples for each subject were divided into "open" vs "closed" sample groups, respectively. For the open group, each of the subjects' plasma samples were immediately aliquoted into sample cups. Sample cups were left open and measured after 0, 15, 30, 60, 120, 240, and 360 mins, respectively. The closed group samples remained in their original tubes stored at 2-8°C; at corresponding time-points, aliquots were taken and measured.
Results:
In general, for the open samples, concentrations for all analytes, except for $HCO3^-$ and Tbil, increased by 9-17% at 360 min ($p<0.003$, Student's *t*-test). This increase is most likely due to water evaporation from the sample, thus, artificially increasing concentration. However, $HCO3^-$ decreased from baseline by 27±2% (average ± SD), $p<0.001$, and TBil trended towards a decrease (9±8%, $p=0.08$) after 360 min. $HCO3^-$ most likely decreased due to CO2 evaporation and TBil trended down in 3 out 5 samples likely due to unprotecting from light. Additionally, the calculated AGAP [Na- (Cl + $HCO3^-$)] increased by 102±16%, $p<0.001$, at 360 min. This large change is amplified by increased Na with concomitant decrease in $HCO3^-$. Even after 15 min, $HCO3^-$ was already significantly decreased (-5±2%, $p=0.007$), with a trend towards an increased anion gap (+16±15%, $p=0.07$). The AGAP was significantly increased at 30 min (+25±15%, $p=0.01$). For the closed system, most analytes had a minimal amount of change that ranged from +/-4% at 360 min. Finally, for the closed system, a 48-hour "add-on" was done. Most analytes had less than +/-10% change. Glu (-14±12%, $p=0.07$) and bicarbonate (-14±15%, $p=0.1$) trended towards a decrease, whereas K (+18±15%, $p=0.05$) and the AGAP (+28±26%, $p=0.09$) trended towards an increase.
Conclusion:
This study demonstrates the rate of change of common plasma chemistries over time in open cups being transported to workstations on an automation line. Our findings show that workflow processes integrated on the automation line may be at risk of releasing falsely elevated/decreased results, *e.g.* when there is a substantial delay in the transfer of open cups to individual measuring modules. We propose that open cups should be discarded if they cannot be assayed within 30-60 min after being processed, depending on the analyte.

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Analysis of Interference on the ADAMS A1c HA-8180V system

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Background and Objective: Important diagnostic and therapeutic decisions are routinely made based on glycated hemoglobin (HbA1c) measurement, which is considered an important indicator of glycemic control in diabetics. The accuracy of HbA1c measurement can be adversely affected by the presence of hemoglobin (Hb) variants. The ADAMS A1c HA-8180V (ARKRAY USA), an 8th generation A1c measurement device from ARKRAY, was recently cleared by FDA. The device measures HbA1c (IFCC mmol/mol and NGSP %) in human whole blood and hemolysate samples using ion exchange high performance liquid chromatography (HPLC). The device features a new column type with an integrated pre-filter that reduces maintenance. Studies have shown the high accuracy and precision (CV ≤ 1%) of the ADAMS HA-8180V system. The purpose of this study was to evaluate the interference of common or known variants, endogenous substances, drugs, and hemoglobin derivatives on the accuracy of A1c measurements by the ADAMS HA-8180V system.
Methodology: An interference study was performed per CLSI EP07-A2 *Interference Testing in Clinical Chemistry*. A hemoglobin variant study was conducted on HA-8180V with 165 samples containing variants A_2, C, D, E, F, or S and compared to results obtained from a reference method free from hemoglobin variant interference. Fifteen (15) drugs, five (5) endogenous analytes, and three (3) hemoglobin derivatives were analyzed by spiking the interferent into two whole blood samples with HbA1c values of ~6.5% and ~8.0%. Ten (10) replicates of each drug/interferent test samples and solvent-only control samples were analyzed using the ADAMS A1c HA-8180V system.
Validation: In the hemoglobin variant study, HbA1c results were found to be accurate (with no significant interference) in samples containing HbA_2 (≤16%), HbC (≤39%), HbD (≤36%), HbE (≤30%), HbF (≤30%), or HbS (≤40%). No significant interference was observed at therapeutic levels up to the highest concentration of fifteen (15) drugs tested: Acetaminophen (20 mg/dL), Acetylcysteine (330 mg/dL), Acetylsalicylic acid (65 mg/dL), Ampicillin (1000 mg/dL), Ascorbic acid (200 mg/dL), Cefoxitin (2500 mg/dL), Cyclosporine (0.67 mg/dL), Doxycycline (50 mg/dL), Ibuprofen (50 mg/dL), Levodopa (20 mg/dL), Metformin (5 mg/dL), Methyldopa (30 mg/dL), Metronidazole (200 mg/dL), Rifampicin (6.4 mg/dL), Salicylic acid (60 mg/dL), and Theophylline (10 mg/dL). Endogenous interferents were tested and found to have no interference at the following concentrations: Albumin (20 g/dL), Conjugated and free bilirubin (100 mg/dL), Rheumatoid factor (750 IU/mL), Triglycerides (2000 mg/dL), Acetylated Hb (50 mg/dL), Carbamylated Hb (25 mg/dL), and Labile Hb (2000 mg/dL).
Conclusion: Studies showed no significant interference with six common Hb variants found in the North American population, fifteen commonly used drugs at therapeutic levels, or eight endogenous analytes and hemoglobin derivatives at physiological lev-

els. The ADAMS A1c HA-8180V system is a robust, safe, and accurate method for routine HbA1c measurement in laboratories.

A-256

Interference of the ClinRep® HPLC Complete Kit for Metanephrines in Urine - A Singapore Hospital Experience.

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Background
Our objective was to evaluate the commercial Recipe®Clinrep® Complete Kit for Metanephrines in Urine on the high-performance liquid chromatography (HPLC) and electrochemical detection system and the suitability of use in our laboratory.
Methods
The HPLC system was Agilent Technologies 1260 Infinity with ClinLab® Digital Amperometric Detector EC3000. The sample collection, storage conditions and sample preparation was performed as per vendor's instruction manual and mobile phase was used as supplied. We collected 39 patient samples over several months and tested in 2 batch runs.
Results
Our sample population consisted of 28 Chinese, 8 Malays, 2 Indians and 1 other, which adequately represents the multiracial proportions of the Singapore society. We found that in 64% of our samples the internal standard was higher than expected. We defined the interference as any multifold increase above 1.35 relative to the internal standard peak height of the calibrator with the respective batches. The interference was chromatographically and electrochemically indistinguishable from the internal standard. Of the different ethnic groups, we found that Indians were most affected (100%), followed by Malays (75%) and Chinese (57%).
Conclusion
The suspected interference is likely an isomer of methoxyhydroxybenzylamine (MHBA), a common ingredient of curry leaves. This dietary interference of the Clinrep® urinary metanephrines kitset was previously reported by Madhawaram and Woollard. Spicy food containing chilies and curry are very common in South East Asian cuisine and are hugely popular across all racial groups in Singapore. This interfering component co-elutes exactly with internal standard and artificially decreases the metanephrines and normetanephrines results. We conclude that this commercial kit is not suitable for use in our population and in our laboratory as it is inconvenient and impractical to ask our patients to adhere to this dietary restriction for at least 24 hours prior to specimen collection.

A-257

Reduction of RF Interference in ELISA by Active and Passive Blocking Agents

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Background: Rheumatoid factor (RF) is a common heterophilic antibody found in rheumatoid arthritis (RA) patients. RF can bind non-specifically to immunoassay antibodies resulting in false signals. This study examines the effectiveness of active and passive blocking agents in reducing RF interference in patient specimens. The specimens were tested across two biomarkers known to be vulnerable to RF interference, Human Cardiac Troponin I (TNNI3) and Human Mucin 16 (CA125). **Methods:** Ten plasma specimens from patients with a RA diagnosis (10 female, age 46-70, RF titer 107->600 IU/mL) were tested in commercial TNNI3 and CA125 ELISA kits per the manufacturers' protocol. Prior to specimen dilution a blocking agent was added directly to the assay diluent. Three passive blockers (mouse IgG, human IgG and goat IgG) and HeteroBlock®, a commercially available active blocking agent, were compared. Passive and active blockers were added to the assay diluent for a final concentration of 200 µg/mL and 20 µg/mL, respectively. Patient specimens were diluted 2-fold immediately prior to testing with and without a blocking agent present in the assay diluent. Four of the ten plasma specimens were tested with 600 µg/mL and 60 µg/mL final concentration of passive and active blockers, respectively. **Results:** Elevated signals were observed for the ten RF-positive plasma specimens prepared without a blocking agent for both the TNNI3 and CA125 ELISA kits. Results are summarized in the table.

Table 1: Results shown as % reduction of signal.

	TNNI3 ELISA		CA125 ELISA	
	% Reduction Average	% Reduction Range	% Reduction Average	% Reduction Range
Human IgG 200 µg/mL	11% (n=10)	0-24%	12% (n=10)	0-24%
Human IgG 600 µg/mL	3% (n=4)	0-6%	7% (n=4)	0-14%
Goat IgG 200 µg/mL	33% (n=10)	11-89%	26% (n=10)	0-93%
Goat IgG 600 µg/mL	4% (n=4)	0-5%	6% (n=4)	1-11%
Mouse IgG 200 µg/mL	42% (n=10)	22-63%	26% (n=10)	0-48%
Mouse IgG 600 µg/mL	42% (n=4)	23-62%	40% (n=4)	14-58%
HeteroBlock 20 µg/mL	76% (n=10)	37-100%	73% (n=10)	33-91%
HeteroBlock 60 µg/mL	100% (n=4)	100%	98% (n=4)	94-100%

HeteroBlock at 60 µg/mL eliminated the interference for the four specimens in the TNNI3 test and reduced the interference below the clinically significant level of 35 U/mL for the four specimens in the CA125 test. **Conclusion:** Passive blocking agents partially reduce interference from heterophilic antibodies like RF. In this study most interference remained even with passive concentrations as high as 600 µg/mL. The active blocking agent demonstrated superior performance at 10% the passive concentration.

A-258

Difference in textbook reference ranges for plasma and serum potassium (K+) is consistent with a purely random K+ component in serum due to clotting: a simulation study

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BACKGROUND: Plasma has long been recommended over serum as the preferred sample type for measurement of potassium (K+), yet use of serum remains prevalent. Deployment of fully automated systems in core laboratories, for which immediate processing of plasma as the default sample type is essential, is an impetus for changing to plasma. However, reference ranges for plasma and serum K+ are different. This is represents a significant change for clinicians, and it is therefore important to convey to users the basis and consequences of the difference. Numerous studies have described the distribution of the difference between serum and plasma K+ across all-comers, attributed to K+ release upon clotting. Our objectives were 1) to demonstrate by simulation that random sampling of a simple difference distribution accounts for the difference in reference ranges between plasma and serum K+; and 2) to discuss the consequences of that difference and how they are important to convey to clinicians**. METHODS**: As a basis for calculations, we used Tietz Textbook reference ranges for plasma (P: 3.4-4.5 mmol/L) and serum (S: 3.5-5.1 mmol/L) that are widely cited by manufacturers in documentation for FDA-approved methods for potassium. Regarding P reference range as the central 95% of a fixed normal distribution, we simulated random sampling of values from plasma, with addition of random sampling of an assumed normal distribution for difference values (D) between plasma and serum, to produce a calculated serum results distribution, C. Appropriateness of an assumed distribution D was assessed simply with respect to whether C reproduced the textbook reference range for serum. Simulations were programmed in Python 2.7.10. **RESULTS**: Simulations using D = 0.31±0.27 mmol/L (average±1sd) is an example for which the calculated distribution C reproduced the textbook reference range for serum. This D corresponds to data of Ladenson et al. (1974, PMID: 4415749): D = 0.31±0.24 mmol/L. Numerous aspects of this demonstration are useful for educational purposes. First, plasma and serum K+ reference ranges are asymmetric in relation to each other rather than simply shifted; viz., lower limits are closer together than are upper limits, as occurs simply because of how reference ranges are defined (central 95% of results). Second, because of the smaller width of P compared to S, a switch from serum to plasma necessarily entails an increase in prevalence of results within any given range of values, given that the increment of reporting (0.1 mmol/L) does not change. The extent of increase will depend on details of the all-comers distribution for K+ at a particular institution. Third, the simulation demonstrates why plasma K+ should be preferred, as any result for serum K+ (and its position relative to S)

is highly imprecise with respect to values for plasma K+ that may have produced it (and their positions relative to P). **CONCLUSIONS**: Simulations demonstrate that textbook plasma and serum K+ reference ranges are consistent with a purely random component of serum K+. The basis and consequences of this relationship, and the advantages of plasma, are important to convey to clinicians when change from serum to plasma is contemplated.

A-259

Quality Control Materials With Extended Availability

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INTRODUCTION:
A foremost advantage of laboratory networks is the harmonization of patient test results. Network laboratory analytical quality control (QC) results can be leveraged to help achieve this goal. To that effect, our healthcare laboratory network, consisting of a Core and of 17 hospital laboratories, uses the same lot of control materials throughout. During monthly QC reviews, this allows detection of significant result clustering that requires further investigation. Bias detection relies on the long-term availability of QC materials of the same lot. Switching from one lot to another is expensive and demanding, even for individual laboratories, and the complexity of a simultaneous switch in several laboratories multiplies this challenge. Here, we describe the validation of chemistry QC materials from a vendor that ensures extended (3 year) lots availability and consolidates six different QC materials into two.
MATERIAL AND METHODS:
Results from Bio-Rad™ (Bio-Rad Laboratories, Hercules, California) QC materials in routine use at the time, were compared with results from QC materials provided by TechnoPath Clinical Diagnostics™ (Tipperary, Ireland). Roche Diagnostics Cobas™ chemistry and immunoassay testing systems in routine use at the Core laboratory were utilized. Two levels of the same QC material were analyzed for 26 routine chemistry tests 15 times over 13 days using the Bio-Rad materials *Multiqual™ Chemistry, Liquichek Immunology™, and Liquicheck IA Plus.™* The results were compared to corresponding results obtained during the same analytical run using two levels of the single TechnoPath *Multichem S Plus™* QC material. 31 - 34 sets of results of two or three levels were obtained for each of 24 different immunoassay tests across 13 days using the Bio-Rad *Liquichek IA Plus™, Liquichek Cardiac Marker™, Liquichek Tumor Marker™* and the *Liquichek Specialty IA Plus™* materials. They were compared with corresponding results obtained with the single *TechnoPath IA Plus™* QC product. In the case of immunoassays, control materials from both manufacturers were tested next to each other, using the same reagent pack and instrument measuring cell.
Calculations:

Imprecision was estimated with the coefficient of variation (CV). The ratios of the TechnoPath CV over BioRad CV were assessed. A ratio of less than 1.0 indicates that the imprecision estimate using the TechnoPath products was lower than that of the corresponding BioRad material.
RESULTS:
Out of the 52 (26 tests x 2 levels) CV ratios obtained from the chemistry controls, and out of 40 CV ratios obtained using two or three levels of immunoassay controls, 28 and 20 were less than 0.9, respectively. Few of these ratios achieved statistical significance in the F-test. Thus, test result imprecision estimates using TechnoPath QC materials were at least equivalent to those using BioRad control results.
DISCUSSION:
The validation was repeated with similar results by each of the participant laboratories using the lot scheduled for routine use. Then, both TechnoPath chemistry and immunoassay QC materials were placed into production. The laboratory network staff is looking forward to a lot change three years from that date, compared with thirteen lot changes in the previous three years.

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Evaluation of the Atellica CH Assays Sigma Metrics

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Background: The purpose of the investigation was to evaluate the performance quality of several Atellica® CH Assays using the Six Sigma philosophy. Six Sigma is a process that uses techniques to identify, quantify, and reduce sources of variability in a product or process. The Sigma metric evaluates the bias between a quality specification and what was observed for that process. This value is expressed in terms of standard deviation (SD). Generally, a Sigma metric of 3 is the minimum quality threshold for a process, and a Sigma metric of 6 or greater is considered to be superior quality. Allowable total error (ATE) is often the quality specification used to evaluate assay performance, as it reflects the maximum error in an assay result that will not change the associated medical decision. Typically, within-lab precision data from quality control (QC) or patient samples—obtained from the assay's instructions for use or historical QC measurements from the laboratory—are used in the evaluation of assay performance. It is common to use a single analyte value, such as a medical decision level, for the calculation. ATE values can be difficult to obtain, as they vary from source to source and may not be available for all assays. For some laboratories, the source of the ATE value is mandated by a regulatory agency, such as CLIA in the U.S. Other sources include the regulatory agencies RiliBÄK (Germany) and RCPA (Australia), a representative clinician consensus, or the biological variable of the analyte. **Method:** There are four inputs required for the calculation of the Sigma metric: analyte concentration, ATE value, estimated bias, and estimated precision at the specified concentration. For the Atellica CH Assays, precision and bias input values were obtained from the respective Atellica CH Assays IFUs. Precision was determined in accordance with CLSI document EP05-A3, where samples were assayed on the Atellica CH Analyzer in duplicate for 20 days. Bias at the selected analyte concentration was calculated using the assay's method comparison data, which was determined in accordance with CLSI document EP09-A3. The CLIA table was the primary source for an assay's ATE. However, if a value did not exist in the CLIA table, other sources that were used included the RiliBÄK table, RCPA table, Ricos Biological Variability, DGKL proficiency survey-acceptance criteria, or CAP proficiency survey-assessment criteria. **Results:** Of the 72 Atellica CH Assays analyzed, 52 assays obtained a Sigma level of >6.0, 20 assays obtained a level between 3.0 and 6.0, and 0 assays obtained a level of <3.0. **Conclusions:** Using the Six Sigma philosophy, all Atellica CH Assays that were analyzed obtained the minimum quality threshold of 3.0 or greater on the Sigma scale, and therefore demonstrateclinically acceptable performance.

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Performance Evaluation of the Serum Indices Feature on the Atellica CH Analyzer

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Background: The Atellica® Chemistry (CH) Analyzer includes an HIL alert feature to notify the operator of potential interference from hemolysis (H), icterus (I), and lipemia (L) in serum and plasma samples. The system automatically displays the recommended HIL alert indices in an assay-test definition if HIL can potentially affect a test result. These indices specify the lowest concentration of hemoglobin, bilirubin, and lipemia that can affect the result. HIL indices are reported with the ALT, AST, LDLP, and UN_c assays as well as a stand-alone HIL test. The objective of this study was to evaluate the analytical performance of the HIL feature. **Method:** Samples were prepared by adding hemoglobin, bilirubin, and INTRALIPID® to normal serum pools. The H and L indices were assigned based on the expected concentrations. The I indices were assigned using the Atellica CH TBil_2 Assay. Seven hemoglobin levels, six bilirubin levels, and nine lipemia levels were prepared and tested for HIL index output using the ALT, AST, LDLP, and UN_c assays and the stand-alone HIL test. Resultant index values were compared to the expected index values. **Results:** For hemolysis, the Atellica CH Analyzer correctly matched the expected values 35 out of 35 times. For icterus, the Atellica CH Analyzer correctly matched the expected values 25 out of 30 times. The 30.0 mg/dL bilirubin sample straddled the third and fourth index buckets, producing the following mean Atellica CH Analyzer results: ALT = 28.8 mg/dL, ALT = 29.1 mg/dL, ALT = 28.7 mg/dL, ALT = 29.2 mg/dL, and ALT = 28.7 mg/dL. For lipemia, the Atellica CH Analzyer correctly matched the expected values 44 out of 45 times. The 2800 mg/dL INTRALIPID sample produced the following mean Atellica CH Analyzer results: ALT = 3014 mg/dL, ALT = 3011 mg/dL, ALT = 3018 mg/dL, ALT = 3003 mg/dL, and ALT = 2980 mg/dL. **Conclusions:** The Atellica CH Analyzer HIL output produced by the stand-alone HIL test and ALT, AST, LDLP, and UN_c assays agrees with the expected values within ±1 index unit.

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Comparison of Results from Paired Specimens Collected in BD Vacutainer Barricor Plasma Collection Tubes and BD Vacutainer PST Tubes

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Background: Conventional plasma separator tubes use a thixotropic gel to isolate plasma from cellular components after centrifugation. Recently, a plasma collection tube which uses a mechanical separator has been marketed for use in the U.S. (Vacutainer Barricor Plasma Collection Tube; BD Diagnostics, Franklin Lakes, NJ). The objective of this study was to conduct a comparison of analytical results obtained from paired specimens collected using Barricor tubes and BD Vacutainer PST tubes, in order to verify whether Barricor tubes are acceptable for the assays investigated in the present report. **Methods:** Using an IRB-approved protocol, healthy volunteer donors (10 female, 10 male; 22-63 years of age) were recruited. Both a PST and Barricor tube were drawn from each volunteer. Collection tubes were processed according to manufacturer instructions. After separation of plasma from cells, testing was performed using fresh samples for a variety of immunoassay and chemistry assays available in our laboratory for which lithium-heparin plasma specimens are acceptable: *cobas c502 & c702* (Roche Diagnostics; Indianapolis, IN): alanine aminotransferase, albumin, alkaline phosphatase, alpha-1 antitrypsin, amylase, aspartate aminotransferase, beta-2 microglobulin, direct bilirubin, total bilirubin, blood urea nitrogen, calcium, carbon dioxide, chloride, cholesterol, complement component 4, c-reactive protein, creatine kinase, creatinine, fructosamine, gamma-glutamyl transferase, glucose, haptoglobin, high density lipoprotein, high sensitivity c-reactive protein, homocysteine, iron, lactate dehydrogenase, lipase, lipoprotein (a), direct low density lipoprotein, magnesium, pancreatic amylase isoenzyme, phosphate, potassium, prealbumin, rheumatoid factor, sodium, soluble transferrin receptor, total protein, transferrin, triglycerides, unsaturated iron binding capacity, uric acid; *cobas e602* (Roche): cancer antigen 125, cancer antigen 15-3, cancer-antigen 19-9, carcinoembryonic antigen, dehydroepiandrosterone sulfate, follicle stimulating hormone, free prostate specific antigen, free thyroxine, free triiodothyronine, human chorionic gonadotropin, luteinizing hormone, myoglobin, parathyroid hormone, PSA, sex hormone binding globulin, testosterone, thyroid stimulating hormone, thyroxine, triiodothyronine, t-uptake; *UniCel DxI* (Beckman Coulter Diagnostics, Brea, CA): bone-specific alkaline phosphatase, estradiol, folate, thyroglobulin, thyroglobulin antibodies, thyroid peroxidase antibodies, vitamin B12; and *Integra* (Roche Diagnostics): ceruloplasmin. Prism (GraphPad Software; La Jolla, CA) and Excel 2010 (Microsoft; Redmond, WA) were used for statistical analysis. **Results:** Direct bilirubin and human chorionic gonadotropin were excluded from analysis due to low analyte concentration in donors. Clinically significant differences (based on desired specification for inaccuracy as listed in the Westgard Desirable Biological Variation Database and/or package inserts) were not observed between Barricor and PST tubes for any of the analytes investigated. Small (but statistically significant, $p<0.05$) differences were observed for several analytes (alanine aminotransferase, alkaline phosphatase, amylase, total bilirubin, calcium, fructosamine, glucose, high density lipoprotein, homocysteine, iron, potassium, unsaturated iron binding capacity, carcinoembryonic antigen, testosterone, and triiodothyronine) although none of these differences would be considered clinically relevant. **Conclusion:** Barricor tubes were verified as acceptable collection tube types for the analyte / instrument combinations investigated in the present report. Future studies should be conducted to verify performance with analyte concentrations encountered in pathologic states.

A-263

Assessment of Panhematin interference in commonly ordered chemistry tests

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Background: Acute intermittent porphyria is estimated to affect about 1 in 20,000 individuals. The porphyrias are a group of eight metabolic disorders, mainly inherited, which result in the accumulation of heme precursors. This accumulation can lead to abdominal pain, an eczema-like rash, and psychiatric symptoms. Complications may include hyponatremia, peripheral neuropathy sometimes causing paralysis, seizures and psychiatric features (PMID: 27982422). Attacks of porphyria may progress to a point where irreversible neuronal damage has occurred (PMID: 200860) and in severe cases liver transplant may be required. Treatment of acute porphyria crises is largely limited to supportive care but may include treatment with hemin (Panhematin), to provide negative feedback inhibition to the heme precursor generation loop. Treatment of the patient with panhematin results in a dark brownish colored plasma. For physicians and labortorians interested in the assessment of the patient's basic metabolism and liver function, concern has arisen for panhematin induced colorimetric interference of spectrophotometric chemistry tests. **Objective:** To evaluate effect of hemin (panhematin) on commonly ordered spectrophotometric tests. **Methods:** To investigate the possibility of panhematin induced spectrophotometric interference of commonly ordered chemistry tests, remnant plasma or whole blood were combined to generate pools of samples spanning the analytical range for 25 colorimetric or immunometric assays on a Cobas 6000 analyzer (Roche, Indianapolis, IN) tested in this study. To these pools, panhematin was added at increasing concentrations of 10, 30, and 100 mcg/mL. The effect of panhematin at 100 mcg/mL on blood gas analysis was also assess using the EG7+ cartridge on an iStat point-of-care (Abbott Diagnostics, Abbott Park, Illinois) analyzer. **Results:** The analyte values of the panhematin-altered samples were compared to the unaltered baseline values via ANOVA. The ANOVA analysis revealed no significant differences in analyte concentration attributable to panhematin. Panhematin similarly had no significant effect on the blood gas parameters measured on the iSTAT analyzer. **Conclusion:** Treatment of patients experiencing acute attacks of porphyria with panhematin does cause the patient's plasma to change color but this color change does not significantly affect lab values produced by a Cobas 6000 analyzer or iSTAT EG7+ blood gas cartridge.

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Methodology Of Creatinine Measurement Can Affect Characteristics And Outcome Pattern In Hospitalized Patients With An Acute Kidney Injury

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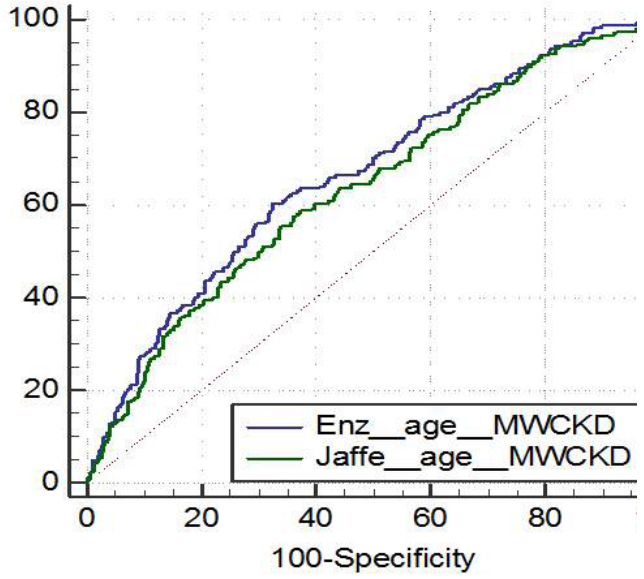
Background: The diagnosis of acute kidney injury (AKI) in hospitalized patients is associated with adverse outcomes. Blood creatinine, the main marker to define AKI, can be measured by the kinetic Jaffé reaction method or an enzyme-based assay. It is not known so far, whether the method for creatinine measurement can affect the diagnosis of AKI. Methods: In a prospective observational study creatinine was measured simultaneously by both assays in patients from a tertiary care hospital using Abbott Reagent Kits™. AKI was diagnosed based on the Kidney Disease: Improving Global Outcomes guideline criteria. Results: From 4590 patients 850 (18.5%) met AKI-criteria by measurement of either assay. 514 (60.4%) were diagnosed by both assays while 168 (19.8%) were detected exclusively or earlier by the Jaffé or enzymatic method respectively. The mean age was significantly lower (66 [56-77] vs 74 [64-79] years) and renal function at admission was significantly better (mean eGFR 83 [65-96] vs 50 [38-68] mL/min/1.73m^2) of AKI patients diagnosed exclusively or earlier by the enzymatic method compared to the Jaffé assay. The AKI stages of patients detected by the Jaffe assay were significantly higher compared to the enzymatic method. The incidence of a composite endpoint including in-hospital mortality and dialysis was similar between both methods (23%). After adjustment for age and renal function the area under the receiver operating characteristic curve for the combined endpoint among AKI-patients detected by either assay was slightly but significantly higher for the enzymatic method in comparison with the Jaffe method (0.665 [0.632-0.697] vs 0.636 [0.603-0.669], Figure). Conclusion: We demonstrate that the cohorts of patients with AKI detected by the respective assay not only differ systematically in basic demographics but also in the risk profile for adverse outcomes.



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Prevalence and diurnal variation of ascorbic acid interference in the macroscopic urinalysis from community and tertiary care patients

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Objective
Investigate the frequency and magnitude of ascorbic acid (Vitamin C) interference in the chemical measurement of blood, glucose, nitrite, and bilirubin in urine specimens from community and tertiary care settings.
Relevance
Ascorbic acid in urine specimens can cause false negative results in the chemical measurement of blood, glucose, nitrite and bilirubin via the dipstick method. The sStated interference occurs at much lower ascorbic acid concentrations for blood (>10 mg/dL for Iris iChem Velocity), than for glucose, nitrite and bilirubin (>300 mg/dL for Iris iChem Velocity). Although this interference is noted, tThere is significant variability in how/if clinical laboratories measure and/or report ascorbic acid, which affects urinalysisand whether the result impacts urinalysis workflow and reporting.
Methodology
This study utilized chemical and microscopic urinalysis results from community and tertiary care patients in Calgary, AB reported between August 1, 2016 and July 31, 2017 (N=529,407). Chemical urinalysis results were obtained from the Iris iChem Velocity. Microscopic urinalysis results were obtained from the Iris iQ200ELITE. Reflexing to microscopic urinalysis is routine for samples with positive protein, nitrite, leukocytes, and/or blood; renal transplant patients; patients <16 years of age; and samples with a cloudy appearance or unusual color. Positivity rates of test results were stratified by ascorbic acid results (negative, 20 mg/dL, 40 mg/dL) and collection site (community, tertiary care, mobile).
Results
Ascorbic acid was detected in 15.3% (n=80,950) of all urinalysis specimens; 10.1% (n=53,675) of patients tested positive for ≥40 mg/dL. Ascorbic acid positivity demonstrated a diurnal variation, with a morning nadir between 0600-0700 and evening peak between 1500-1700. The positivity rates for blood, glucose, nitrite and bilirubin in positive (20 mg/dL, 40 mg/dL) vs. negative ascorbate specimen groups showed statistically different patient distributions for all of these tests (p<0.01). However, chemical analysis of blood showed the greatest difference (>3 fold): 25% of ascorbate negative specimens gave positive blood result versus only 8% of ascorbate positive specimens being positive for blood. As all positive macroscopic blood results reflexed for microscopic urinalysis, this threefold difference could have obstructed 48,965 specimens from undergoing microscopic analysis, with ~2,099 of these specimens potentially confirming positive for blood by macroscopy; this calculation is based upon the microscopic positivity rates in specimens positive for blood, but negative for ascorbate.
Conclusions
Ascorbic acid positivity rates follow diurnal variation and can result in a significant interference in the chemical measurement of blood by the dipstick method. This can prevent a significant proportion of urine specimens from reflexing to microscopic analysis if blood positivity is used as an automated reflex rule.

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TIBC and TSAT calculation cancellation in the presence of elevated ferritin: Are we misinterpreting Tietz' study?

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Objective
Determine if elevated ferritin concentrations result in a significant difference in the measured concentration of iron, total iron binding capacity (TIBC) and percent transferrin saturation (TSAT) in a community patient population.
Relevance
Elevated ferritin concentrations indicate iron overload. A comprehensive evaluation of an iron overload state requires the additional determination of iron, TIBC and TSAT. It has been reported that elevated ferritin concentrations >1200 µg/L interfere with the measurement of iron by ferrozine, and this is a method limitation for calculating TIBC and TSAT (Roche Diagnostics).
Methodology
Data was procured for all ferritin, transferrin, iron, unsaturated iron binding capacity, TIBC and TSAT testing for specimens collected at an outpatient centre in Calgary, AB (September 15, 2016 - May 31, 2017). The data set contained 82,883 unique results from 73,697 patients. Data analysis included the Mann-Whitney U test and Kruskal-Wallis test.
Results
Patient groups were stratified by ferritin concentration [>1200 µg/L (n=315), 401 -1200 µg/L (n=4500), 30-400 µg/L (n=63,852), and <30 µg/L (n=14,215)]; they were statistically significantly different for iron, TIBC and TSAT. Elevated ferritin stratifications [>1200 µg/L; 401-1200 µg/L] yielded a statistical difference in iron [24.0±10.4µmol/L vs 19.1±7.4µmol/L] (p<0.01) and TSAT [0.50±0.23 vs 0.38±0.15] (p<0.01), yet no statistical difference for TIBC [48.5±9.7µmol/L vs 50.8±8.0µmol/L] (p=0.08).
Conclusions
The observed pathophysiological correlation between elevated ferritin and iron concentrations, alongside no significant statistical difference in calculated TIBC indicates that the laboratory can report Iron, TIBC and TSAT when ferritin is >1200 µg/L, but results should be interpreted with caution.

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Impact of different processing and storage conditions on Epithelial growth factor (EGF), Osteopontin (OPN), and Uromodulin (UMOD) in urine.

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Background: Urine Epithelial growth factor (EGF), Osteopontin (OPN), and Uromodulin (UMOD) have been shown to be associated with several acute and chronic kidney diseases. Urine samples are typically collected, processed, and stored with different procedures prior to analysis. However, to be clinically useful, identifying potential variations in these biomarkers arising from sample processing and storage prior to analysis is important.
Objective: To determine the effects of four sample handling procedures on the concentration of EGF, OPN, and UMOD.
Method: We processed urine samples from 20 participants immediately after collection using the following four methods: A) Centrifuged (10 min at 1000 rpm at 4°C). B) Centrifuged and stored at 4°C for 48h. C) Centrifuged and stored at 25°C for 48h. D) No centrifugation. All samples were immediately stored at -80°C until analysis. We analyzed all samples (n=80) using the Kidney Injury Panel 5 (KIP-5) panel available from Meso Scale Discovery (MSD), which is a sandwich immunoassay platform requiring a lower sample volume than standard ELISA. We performed all testing on a single plate to eliminate inter-assay variation. We used procedure (A) as reference since this is the current research standard for processing urine samples. We calculated correlation (r^2), concordance correlation coefficient (CCC), and inspected procedural bias via Bland-Altman analysis for each pair.
Results: R^2, CCC, and procedural bias results are summarized in the table below.
Conclusion: Our findings demonstrate that storing urine specimens at 4°C up to 48h following centrifugation should not significantly affect EGF and UMOD measurements. However, samples should be centrifuged and stored immediately at -80°C for measurement of OPN. There is a considerable effect on OPN and UMOD levels related to centrifugation of specimens and storage at room temperature.

Table. Description of study procedures, correlation, concordance, and mean bias for each procedure

Group	A	B	C	D
Centrifugation	Yes	Yes	Yes	No
Temporary storage	None	4°C for 48h	25°C for 48h	None
EGF	Ref.	0.99, 0.99, -0.9%	0.99, 0.99, -8.6%	0.99, 0.99, -4.1%
OPN	Ref.	0.99, 0.98, -15%	0.81, 0.69, -65%	0.86, 0.92, 0.21%
UMOD	Ref.	0.98, 0.96, -4.7%	0.87, 0.83, -21%	0.79, 0.79, 31%

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US Laboratories Proficiency Testing Performances from 1994-2016: Results reported to the Centers for Medicare & Medicaid Services

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Background:
The Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations require laboratories performing nonwaived patient testing to perform proficiency testing (PT) for specified analytes in Subpart I of CLIA regulations. For these analytes, laboratories participate in PT events three times annually. Typically, each event has five challenge samples, which are scored using acceptance criteria published in the CLIA regulations. Laboratories must achieve a score of at least 80% (4 of 5 acceptable results) for a "satisfactory" event score. This study aims to extend a previous PT performance evaluation from 1994-2006[1] with additional data from 2007-2016.
Methods:
We used CLIA data from the Centers for Medicare & Medicaid Services Quality Information and Evaluation System database from 1994-2016. Using similar methodology,[1] we categorized laboratories required to perform PT prior to implementation of CLIA (i.e.hospital and independent) as HI, and laboratories previously unregulated by CLIA (i.e. all other testing sites) as AOT. Approximately 12,000,000 PT events were analyzed overall, performed by an average of 33,531 laboratories each year (roughly 9,000 HI and 23,000 AOT laboratories annually). We compared unsatisfactory PT event rates of HI and AOT laboratories for 15 analytes: alanine aminotransferase, amylase, bilirubin, cholesterol, digoxin, glucose, hemoglobin, leukocyte count, potassium, prothrombin time, theophylline, thyroxine, triglycerides, white blood cell differential, and uric acid. PT events associated with reason codes (e.g. non-performance of PT, ungradable PT) were excluded since scores would not reflect analytical performance of the testing sites. A Mantel-Haenszel test was used to calculate odds ratios for HI compared to AOT.
Results:
Unsatisfactory PT performance for fourteen analytes decreased from 1994-2016 for both HI and AOT laboratories. In 1994, unsatisfactory PT rates ranged 0.94%-4.07% and 4.82%-11.72% for HI and AOT laboratories, respectively. By 2006, HI rates ranged from 0.27%-1.98% and AOT rates ranged 0.67%-4.86%. By 2016, rates decreased to 0.26%-2.21% for HI and 0.49%-2.97% for AOT. Rates increased for only one analyte (white blood cell differential) among HI laboratories, by 1% from 1994-2016. The odds ratios of unsatisfactory rates between HI and AOT laboratories ranged from 2.57-11.2 in 1994, and decreased to 0.86-2.51 by 2016.
Conclusions:
Since CLIA implementation, improvements in PT performance were greater for AOT than HI. PT requirements for HI laboratories existed prior to CLIA and may explain the initial large differences from AOT. AOT laboratories made substantial improvements in PT performance and are becoming comparable to HI laboratories. Improved PT performance for both laboratory categories are most likely multifactorial and may be attributable to CLIA regulations, improved technology, and staff experience performing PT; thus, the impact of these and other factors on PT performance should be explored further. The results presented in this study is one way of evaluating the data. Future studies may evaluate the data set with different variables and analyses providing a new perspective.
1. Arch. Pathol. Lab. Med. 2010;134:751.

A-269

5-day storage and sedimentation partially affect routine analyte stability for clinical studies and biobanking

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Background: Specimen collection, processing, receiving, and retrieval are key processes for biobank sample quality, that have recently been implemented in the latest ISBER guidelines. While quality is always defined for a specific purpose, preanalytical efforts increase massively with speed and cooling levels. We investigated, whether high-throughput processing and intermediate storage in a fully automated clinical chemistry lab is sufficient to maintain the quality of routine analytes for clinical studies and biobanking.
Methods: We randomly collected 20 patients' leftover sera after routine analysis. From each sample three layers from the top to the bottom were extracted on day 1, 3, and 5. Following analytes were measured: potassium, sodium, protein total, TSH, FT3, FT4, HDL cholesterol, triglycerides, lipase, and IgG. During these 5 days the samples were refrigerated at 4 °C. All analytes were determined on a Roche™ Cobas 8000 analyzer. We used generalized linear mixed effects modeling with analyte levels as dependent, storage days and layers and their interaction as fixed and patient pseudo-ID as random effects.
Results: We found small effects of measurement day for potassium and protein, lipase, HDL, and sodium. After multiple testing correction, only potassium and protein remained significant.
Conclusion: Levels of the tested analytes with exception of potassium and protein are stable when automatically refrigerated for up to 5 days in clinical routine. Even for sedimentation-prone analytes (e.g. lipids, protein) no significant change or differences between layers could be observed. While the effect sizes are very small (e.g. <3% for potassium), the statistical significance does not reflect clinical relevance - nevertheless, for large scale evaluations this minute effect should be kept in mind. We conclude that processing and up to 5 days intermediate storage on a total lab automation system (TLA) is sufficient to maintain the quality of the aforementioned routine analytes for clinical studies.

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Assessing the impact of biotin and simulating patient risk using the Elecsys Troponin T Gen 5 STAT assay

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Background: Recent attention has focused on erroneous immunoassay results due to biotin interference. We previously conducted a study measuring biotin concentrations in plasma samples from patients in the Emergency Department (ED) to assess the prevalence of potentially interfering biotin concentrations. One concern within the ED setting is biotin interference with critical tests such as Troponin T. In our previous study, 1.7% of patient samples had biotin concentrations ≥20 ng/mL (range: 20-280 ng/mL) which is the manufacturer's claim for biotin interference with the Elecsys Troponin T Gen 5 STAT immunoassay (Gen5 TnT, Roche Diagnostics, Inc.). In the current study, we verified the biotin interference threshold for the Gen5 TnT assay and used simulation based upon observed biotin concentrations in ED patients to assess the risk of clinically significant interference for the Gen5 TnT assay. Methods: Biotin interference studies were performed by adding varying concentrations of biotin (Sigma-Aldrich) to plasma pools with three target TnT concentrations (low: 10-13 ng/L; medium: 40-42 ng/L; high: 177-194 ng/L). Plasma was mixed with biotin stock solution (9:1, high biotin pool) or saline (9:1, zero pool). The zero and high biotin plasma pools were mixed to create eleven samples (0-200 ng/mL biotin). Samples were analyzed on the Roche Cobas® e602 and e411. Clinically significant bias, defined as change in result of 4 ng/L or 10%, was used to determine biotin interference thresholds. Using biotin concentrations previously measured in specimens from ED patients and applying linear regression analysis equations from each biotin interference experiment on the e411 (STAT lab analyzer for ED specimens), the proportion of patients at risk for clinically significant decreases in hypothetical TnT results was simulated. Results: The biotin interference thresholds for the low, medium, and high TnT pools were 100 ng/mL, 20 ng/mL, and 40 ng/mL, respectively, for the e411 and 120 ng/mL, 40 ng/mL, and 20 ng/mL, respectively, for the e602. The simulation data revealed the percentage of patients (of 1442 total samples originally studied) who would have had clinically significant decreases in TnT results due to biotin interference (range of false decrease) at the following hypothetical TnT concentrations: 13 ng/L, 0.2% (4-9 ng/L decrease); 40 ng/L, 0.8% (4-34 ng/L decrease); 177 ng/L, 0.4% (20-169 ng/L decrease). Conclusions: The threshold for biotin interference on the Gen5 TnT assay varies de-

pending on TnT concentration and analyzer model (e411 vs. e602). Based on our findings, up to 0.8% of ED patients would be at risk for clinically significant decreases in TnT values of at least 4 ng/L due to biotin interference. Importantly, in our patient population biotin was present at concentrations that would have caused elevated TnT values to erroneously fall within the sex-specific reference intervals. These findings illustrate the magnitude of biotin interference with the Gen5 TnT assay and highlight a patient population at risk for potential harm.

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Verification of Hemolysis Interference Claims for 31 Common Chemistry Analytes on the Beckman AU 5800

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Background: Hemolysis is the most common reason for rejecting samples in clinical laboratories because it can be a significant source of measurement error. Since the interference data provided by assay manufacturers is often instrument or reagent dependent, and the level of detail provided to the consumer may vary, it is important to verify the effect of hemolysis on each laboratory test prior to implementation. The objective of this study was to evaluate the presence, direction, and degree of interference from hemolysis on 31 common chemistry assays on the Beckman AU5800 chemistry analyzer. The data obtained was used to establish appropriate specimen rejection criteria when clinically significant interferences were observed and to prevent unnecessary rejections. **Methods:** Hemolysate was purchased from Sun Diagnostics (New Gloucester, ME). An initial comparison study was performed between pooled patient specimens and matrix-matched Bio-Rad QC material with similar target concentrations to determine commutability for interference experiments. Two concentrations of commutable QC materials for each analyte were then spiked with varying amounts of hemolysate corresponding to the hemolysis indices on the AU5800. Deviation from the unspiked QC results was assessed for each analyte using CLIA limits or a difference of 10% as recommended in the AU5800 IFU assay-specific interference criteria. **Results:** The results obtained for the pooled patient samples were in agreement with the matched QC material. 14 of 31 analytes tested in the low level QC demonstrated interference exceeding the defined acceptable limit for that analyte. However, only 6 of 31 analytes tested in the high level QC exceeded the defined acceptable limit for the particular analyte. **Conclusions:** This study defined the degree and directionality of interference from sample hemolysis for 31 common chemistry analytes at various concentrations on the Beckman AU5800. For some assays, there was differential impact of hemolysis at low versus high analyte concentrations.

Low QC				High QC			
Analyte	Analyte concentration	Hgb conc (mg/dL)	Interference Directionality	Analyte	Analyte concentration	Hgb conc (mg/dL)	Interference Directionality
Ammonia	53.5 (umol/L)	≥ 50	↑	LDH	89 (U/L)	≥ 50	↑
D. Bilirubin	0.3 (mg/dL)		↓	D. Bilirubin	2.5 (mg/dL)		↓
Haptoglobin	67.5 (mg/dL)		↓	Haptoglobin	225 (mg/dL)	≥ 100	↓
Digoxin	0.5 (ng/mL)		↓	Potassium	7.5 (mmol/L)		↑
LDH	311 (U/L)	≥ 100	↑	Digoxin	3.0 (ng/mL)	≥ 300	↑
Potassium	2.7 (mmol/L)		↑	Ammonia	325.5 (umol/L)		↑
Phosphorus	1.9 (mg/dL)		↑				
Cholesterol	104.0 (mg/dL)	≥ 200	↑				
Lipase	16.5 (U/L)		↑				
Magnesium	1.1 (mg/dL)		↑				
GGT	24.5 (U/L)		↓				
Vancomycin	5.9 (ug/mL)		↓				
Albumin	2.4 (g/dL)	≥ 300	↑				
ALP	26.5 (U/L)		↓				

Table 1. Hemolysis Interference Study Results

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Evaluation of the effect of non-fasting on the lipid levels

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Background: The recommendation of fasting for the collection of laboratory tests is a common practice in health services and aims to reduce biological variability. However, since the patient spends most of the day fed, lipoprotein dosing without fasting could better portray the individual's metabolic status. Studies have shown that the lipid profile without fasting is better or at least equivalent in predicting cardiovascular risk. **Objective**: to evaluate the impact of fasting time in relation to the values of the lipid profile of patients attended at a university hospital. **Methods:** Serum samples from patients were obtained between September 2017 and February 2018 and processed in VITROS 5,1 FS and 5600 (Ortho Clinical Diagnostics, USA) for total cholesterol, HDL, LDL and triglyceride dosage. Next, the results were stratified by fasting time (hours). The SPSS Statistics 23 program (IBM, USA) was used to calculate the mean, confidence interval and statistical tests (Mann Whitney and Kruskal Wallis). The significance level was set at 0.05. **Results:** 10,798 total cholesterol, 9,253 HDL, 9,109 LDL and 11,190 triglycerides were measured. Table 1 shows the results of the lipid profile by fasting time. Statistical difference in the comparison of the results by fasting time ($p < 0.05$) was observed for all the analytes. However, no significant difference was observed between the confidence intervals of the mean values. **Conclusion**: The difference found between the means of the results in relation to the fasting time did not present clinical significance and was found to be smaller than the biological variability expected for the tests. Additionally, confidence intervals overlapped with one another. This study helps to reinforce the findings in the literature and the recommendation to flex the fasting to measure the lipid profile.

Table 1. Lipid profile according to the fasting time

Fasting time (hours)	Total Cholesterol		HDL Cholesterol		LDL Cholesterol		Triglycerides	
	n	mean (95% CI)	n	mean (95% CI)	n	mean (95% CI)	n	mean (95% CI)
01	148	176.0 (167.7-184.3)	129	48.8 (45.4-52.2)	126	98.1 (90.6-105.7)	197	158.4 (132.1-184.7)
02	65	172.8 (163.3-182.3)	51	49.0 (44.5-53.5)	48	96.3 (86.4-106.1)	72	166.8 (137.4-196.2)
03	80	177.3 (168.7-186.0)	71	51.4 (48.1-54.8)	70	93.6 (86.2-101.0)	84	159.1 (135.5-182.7)
04	89	177.6 (199.9-188.0)	81	46.2 (52.9-48.5)	78	103.0 (123.9-111.2)	97	152.2 (213.3-161.1)
05	50	179.3 (165.1-193.5)	41	46.0 (40.0-51.9)	39	99.5 (85.6-113.4)	65	234.9 (90.7-379.1)
06	55	177.0 (159.5-194.5)	51	48.5 (42.6-54.4)	51	104.4 (91.0-117.8)	66	163.3 (127.1-199.6)
07	20	169.4 (150.9-187.9)	18	49.8 (44.2-55.4)	18	94.5 (76.9-112.1)	20	136.2 (108.0-164.4)
08	1766	186.9 (184.6-189.1)	1679	53.2 (52.4-53.9)	1647	105.0 (103.2-106.9)	1785	141.6 (137.2-146.1)
09	1268	182.9 (180.4-185.4)	1060	52.5 (51.5-53.6)	1048	102.9 (100.6-105.2)	1306	149.3 (134.1-164.5)
10	1554	182.9 (180.8-185.0)	1289	52.4 (51.5-53.4)	1271	103.6 (101.6-105.6)	1597	142.2 (136.5-147.9)
11	1178	182.6 (180.1-185.0)	1036	52.5 (51.5-53.4)	1025	103.6 (101.4-105.8)	1205	137.8 (132.5-143.1)
≥ 12	4525	186.1 (184.7-187.5)	3747	51.1 (50.6-51.7)	3688	105.1 (103.9-106.4)	4696	152.2 (147.2-157.2)

CI: confidence interval for the mean.

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Acceptability Varies: Lipid Interference in the Siemens BNII Nephelometric Assays

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Background: Nephelometry is used to quantitate many different serum proteins by forming antigen/antibody complexes and measuring the light scatter produced by these complexes. Lipemia is a known interferent in nephelometry testing, causing erroneously high results. Package inserts indicate that lipemic samples should not be run, but exclusion criteria are not well defined. Visual detection of lipemia by labora-

tory staff can result in highly variable outcomes as rejection of samples may reflect interpersonal bias. Determining the acceptability of lipemic samples is a time-consuming process for technologists, with 275 recognized per month of 48,000 total tests. The purpose of this study was to determine the impact of lipids on nephelometry and to establish values for sample rejection, which affect 0.5% of all samples received by our laboratory. Methods: Lipid interference testing was performed on the Siemens BNII nephelometer for alpha-1-antitrypsin (AAT), anti-streptolysin O (ASO), anti-DNAse B (ADNAS), prealbumin (PALB), albumin (ALB), C1 esterase inhibitor concentration (C1ES), complement C3 (C3), complement C4 (C4), and beta-2 microglobulin (B2M) tests (all by Siemens Healthineers) using residual waste serum samples from routine laboratory testing. Samples with normal and abnormal concentrations of each analyte were selected and spiked with 1600 mg/dL of lipid solution (Intralipid®). Further studies using smaller amounts of Intralipid were performed if required to meet acceptance criteria (analyte recovery between 80-120%). Results: The assays which use routinely a starting dilution of 1:400 (B2M, ALB, ASO) demonstrated acceptable performance (80-120% recovery) at 1600mg/dL Intralipid®. Tests with a starting dilution of 1:20 or 1:5 (AAT, PALB, C1ES, C3, and C4) were predictably more sensitive to lipid interference, with recoveries from 127-266% at 100 mg/dL added Intralipid®, which is the lowest amount of lipemia to be distinguished visually by technologists. The C1ES and C4 assays are particularly susceptible to lipid interference due to the low starting dilutions and reporting ranges down to 3 mg/dL. Addition of 40 mg/dL Intralipid® increased the recoveries of abnormal C1ES and C4 samples to 127 and 160% respectively. Conclusion: Tests that run at 1:20 and 1:5 dilutions on the BNII platform are very sensitive to low levels of lipemia interference. Assay-specific studies are required to determine thresholds for sample acceptability and remediation of lipid interference, and these results suggest that a universal cut-off is not appropriate for nephelometry testing. Process improvement measures include education for technologists performing these assays and implementation of turbidimetry standards to aid visual inspection of samples. Ideally, measurement of a lipemia index using an automated chemistry analyzer would be required for assays where visually non-apparent lipemia < 100 mg/dL is a problem, such as the case for C1ES and C4.

A-274

Biotin leads to invalid results in a urine hCG immunochromatographic assay but not in a urine fluorescent immunoassay

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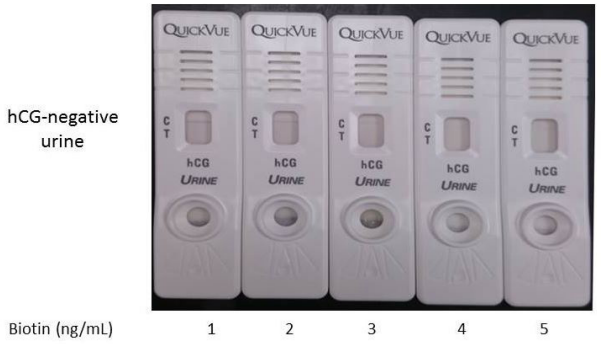
Background
Biotin supplementation has become increasingly popular in recently year, and is commonly administered at higher than recommended daily dose of 300 mg. The biotin-streptavidin system is widely used in clinical laboratory immunoassays, and high blood biotin concentrations have been shown to interfere with a number of immunoassays. This study reports the interference of biotin on a urine human chorionic gonadotropin (hCG) immunochromatographic assay but not in a urine fluorescent immunoassay.
Methods
Three patients taking biotin supplementation and had invalid results (control line not showing) on the QuickVue one-step hCG urine test (Quidel Corporation) were studied at Texas Children's Hospital Pavilion for Women. Urine samples were also tested on Sofia urine hCG fluorescent immunoassay (Quidel Corporation) for comparison. Biotin (Sigma-Aldrich) was used to spike into biotin-free urine.
Results
Patients reported biotin intake corresponding to blood concentration of 5 ng/mL, which was reported to have impact on blood immunoassays and supports our hypothesis of biotin interference in urine hCG immunoassay after ruling out causes from protein or lipid. Serial solutions of biotin were spiked into biotin-free urine (serum hCG <2.4 mIU/mL) with final biotin concentrations of 1, 2, 3, 4, and 5 ng/mL. Results revealed that interference to the manual QuickVue immunochromatographic assay becomes evident at 4 ng/mL (Figure). Retesting of patients' urine samples on Sofia urine hCG fluorescent immunoassay showed negative results (< 20 mIU/mL hCG) with no intereference found.
Conclusion
The study indicates that biotin most likely intereferes with the control line in the manual QuickVue one-step hCG urine test in patients with negative serum hCG levels, while Sofia urine hCG fluorescent immunoassay is not subjective to this interference. It raises the awareness that biotin may not only interefere with blood-based immunoassays but also with urine immunoassays using the biotin-streptavidin system.



A-275

Feasibility of using same serum/plasma sample tubes for HCV antibody and reflex HCV RNA testing

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Background: Current CDC guidelines for the diagnosis of hepatitis C recommend testing for HCV RNA for all individuals who test positive for antibodies to HCV (anti-HCV) with an aim to identify current HCV infections. To avoid the potential for cross-contamination, the same sample tube used for anti-HCV testing on various automated serology platforms is not used for HCV RNA detection and instead a pristine sample is required which poses a challenge in full implementation of the reflex testing algorithm. Objective: To evaluate the potential of cross-contamination of HCV RNA-negative samples and determine the feasibility of using same serum/plasma samples (single tube) for HCV RNA detection that were used for anti-HCV testing on various automated serological platforms. Methods: A panel of 10 HCV RNA positive plasma samples with HCV RNA levels ranging from 2.15 $\log_{10}$ IU/ml to 7.74 $\log_{10}$ IU/ml and 10 HCV RNA negative samples were tested for anti-HCV on four automated serology analyzers: Ortho-Clinical VITROS ECI, Abbott ARCHITECT, Roche Elecsys, and Siemens ADVIA Centaur XPT. HCV RNA-negative samples were interspersed in between HCV RNA positive samples for anti-HCV testing in 3 separate batches of 20 samples. Following anti-HCV testing, the HCV RNA-negative samples were retested for HCV RNA in duplicate by Roche COBAS Ampliprep/Cobas Taqman HCV v2.0. Results: Of the total of 20 samples, 7 were positive for anti-HCV on all four automated serology platforms. None of the HCV RNA-negative samples tested for anti-HCV on the Vitros ECI, Elecsys, and the ADVIA Centaur XPT platforms showed false positive HCV RNA results on reflex testing. Over the triplicate runs, 7 of the 10 HCV RNA negative samples tested positive a single time after testing for anti-HCV on the ARCHITECT platform. HCV RNA levels in these false-positive samples were low (<15 IU/mL). Conclusion: There was no evidence of cross-contamination of HCV RNA-negative samples tested for anti-HCV on three of the four automated platforms equipped with a system that disposes of pipette tips after they come in contact with serum/plasma samples. The ARCHITECT machine, the only platform that uses a fixed probe strategy for sampling followed by its washing, was the only one shown to introduce cross-contamination of HCV RNA-negative samples. Reflex testing of anti-HCV positive samples for HCV RNA using the same sample tube is likely feasible for all automated platforms that use disposable tips for sampling.

A-276

Normalization of Newborn Screening Laboratories MS/MS Analyte Results and Cutoffs Using the CDC NSQAP Reference Materials.

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Background: Lab policies and protocols for newborn screening reporting vary from state to state. This variability could mean that a child that might be identified and treated in one state, might be missed and suffer serious neurologic sequelae in another. Newborn screening laboratories cannot accurately compare mass spectrometry-derived analyte results and cutoff values due to differences in testing methodologies (i.e., derivatized vs. non-derivatized methods). The Center for Disease

Control and Prevention's (CDC) Newborn Screening Quality Assurance Program (NSQAP) provides newborn screening labs with quality control (QC) materials which contain endogenous and three enriched levels of amino acid and acylcarnitines, as well as Proficiency testing (PT) materials that mimic analyte concentrations of newborns with metabolic disorders. The objective of this study was to normalize US and international newborn screening laboratory results and cutoffs using the NSQAP QC samples, and validate normalization using the PT specimen results. **Methods:** NSQAP QC and PT data reported from US and international laboratories in Q3-2016, Q1-2017 and Q3-2017 were used in this study. QC materials were provided as dried blood spot cards which included a base pool and the base pool spiked with specific concentrations of metabolites in a linear range. For each laboratory, the CDC NSAQP QC quantified metabolite values were regressed on each laboratory's quantified QC metabolite values using Deming regressions. The regression parameters were used to normalize the values of all metabolites in PT materials and cut-offs from the different labs to the same scale based on CDC results and cutoffs. The %RSD was calculated for the raw and normalized PT data for comparison between and across metabolites. **Results:** Regression parameters were calculated for 17 acylcarnitines and 8 amino acids. Across the three quarters, all laboratory reported PT metabolite values had decreased %RSD after normalization using the NSQAP QC materials. In Q3-2016 results, overall the %RSD decreased from 0.3 up to 3.0-fold. The largest method associated decrease in %RSD for the US laboratories was malonylcarnitine, citrulline, glutarylcarnitine, and succinylacetone which had %RSD decreases from 56.7% to 18.7%, 15.8% to 6.63%, 31.7% to 14.6%, and 50.9% to 24.5%, respectively. Bias plots were created to visualize method differences between laboratory results and cut-offs before and after normalization. Some analytes exhibited significant differences between raw PT values obtained by different MS/MS methods, but after normalization these PT values were homogeneous. The results of this study could assist newborn screening labs compare analytical results, cutoffs, and healthy population range differences to facilitate uniformity. **Conclusions:** Inter-laboratory newborn screening analyte results and cutoffs can be normalized by using external QC materials.

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Frequency of hemolysis in potassium samples collected during cardiovascular surgery

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Background: Hemolysis is a common cause of pseduohyperkalemia. For this reason, serum and plasma samples with hemolysis greater than the manufacturer-defined H index cut-off are recollected in our practice. Arterial blood gas specimens, collected intraoperatively and with requests for whole blood potassium measurement, are often visually hemolyzed upon separation of plasma from whole blood. We studied whether the H index cut-off used for serum and plasma samples in our practice would be appropriate for determining acceptability of whole blood samples for potassium measurement. Methods: Arterial blood gas samples were collected in Portex syringes (Smith Medical) containing electrolyte-balanced lithium heparin by OR staff, mostly during cardiovascular surgery, and sent immediately to the laboratory for potassium measurement on a Radiometer ABL90 blood gas analyzer (Radiometer America). After separation of lithium heparin plasma in an Eppendorf Mini-Spin centrifuge (Eppendorf AG), samples that were visibly hemolyzed had H index measured on a Roche Cobas c501 analyzer (Roche Diagnostics). Primary analysis consisted of mean ± SD H index, number/percent of samples with H index below an H index of 125 (cut-off for serum/plasma specimens), and correlation between H index in visibly hemolyzed blood gas specimens and whole blood potassium concentration. Results: Approximately 1400 blood gas samples were collected intraoperatively, of which 36 samples were judged to have visible hemolysis. The mean (±SD) H index was 244 ± 89. Only 2 of 36 blood gas samples had an H index less than 125, which is the defined cut-off for serum/plasma potassium on the Roche Cobas c501 analyzer (Roche Diagnostics). There was a weak relationship between H index and whole blood potassium among visibly hemolyzed blood gas samples. The slope of the relationship was 0.0026, with an r^2 of 0.13. The Pearson correlation coefficient was 0.36. Biologic variability of an individual patient's potassium values, as determined by comparing results from patients that had serial testing within the surgical procedure, was much greater than any effect that could be attributed to sample hemolysis. Conclusion: Blood gas samples collected intraoperatively in cardiovascular surgery are often visibly hemolyzed. Application of an H index cut-off derived for serum and plasma samples does not seem appropriate; as the relationship between H index and whole blood potassium is weak among visibly hemolyzed samples and biologic variability in potassium is much higher than any effects of hemolysis. We speculate that free hemoglobin levels in samples collected during cardiovascular surgery are higher due to elements of the procedure such as the bypass circuit, cell salvage devices, or cooling of the patient.

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Lack of Harmonization of IGF-1 Assays Calibrated with Materials Traceable to WHO International Reference Reagent 02/254

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Background: Measurement of insulin-like growth factor 1 (IGF-1) is crucial in the diagnosis of growth hormone (GH) related diseases. Failure to diagnose and treat GH deficiency in neonates and children leads to short stature, delayed development, and metabolic disorders later in life. Hence, it is critical to accurately measure IGF-1 levels and ensure the harmonization of IGF-1 test results across different methods and institutes. In an effort to harmonize IGF-1 assays, the WHO established IRR 02/254. We sought to assess the harmonization of assays calibrated with material traceable to IRR 02/254. **Methods:** After obtaining institutional review board approval and informed consent, we compared IGF-1 measurements in 111 patient samples between the Liaison (DiaSorin) and Immulite 2000 (Siemens) immunoassay methods. We also compared the results from the immunoassays to IGF-1 levels measured in 61 of the 111 samples using liquid chromatography/mass spectrometry (LC-MS). Comparisons were carried out using Deming regression analysis. No significant deviations from linearity were found using the Cusum test for linearity ($P > 0.05$). **Results:** Good overall agreement was observed in the comparison between IGF-1 measurements by LC-MS and the Liaison methods with a slope of 0.98 (95% CI 0.87, 1.09) and an intercept of -16.28 (95% CI -32.92, 0.37). IGF-1 concentrations measured using the Immulite 2000 method showed a proportional negative bias compared to both the Liaison and LC-MS methods with slopes of 0.75 (95% CI 0.68, 0.81) and 0.76 (95% CI 0.69, 0.83), respectively. We then prepared three levels of IRR 02/254 following instructions in the package insert. We measured IGF-1 in the three levels of IRR 02/254 by the Liaison, Immulite, and LC-MS methods and compared the results against the patient comparisons. At the highest level of IRR 02/254 IGF-1 tested (772 ng/mL), the measured IGF-1 value fell outside of the 95% confidence interval for the patient comparison, suggesting that this level of IRR 02/254 may not be a commutable standard. **Conclusion:** Test harmonization depends on the commutability of reference materials (RM), which is the closeness of agreement of the mathematical relationship between the measurements of RMs and patient samples using multiple methods; a RM with good commutability will behave similarly to patient samples between different assays. Method calibration using non-commutable RMs leads to significant discrepancies between interassay test results. In sum, these data suggest a lack of harmonization in the IGF-1 assays tested here.

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Are patients adequately informed about procedures for 24-hour urine collection?

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Background: Traditionally, the quantification of several analytes requires a 24-hour collection of urine as their excretion may vary with posture, physical activity, protein intake and hemodynamic factors among others. This method has many inconveniences being frequently unreliable because of errors in collecting a complete 24-hour urine sample as it requires the direct participation of the patient. Any failure to complete the 24-hour urine collection can lead to incorrect tests results and possible diagnostic errors. The aim of this study was to evaluate the patients' knowledge about the procedures to adequately collect 24-hour urine samples and the importance of following the guidelines provided by the laboratory. **Methods:** We interviewed 158 randomly chosen outpatients who attended the laboratory with collected 24-hour urine sample and agreed to respond to the proposed questionnaire. This questionnaire was anonymous and consisted of 18 questions that dealt with personal opinions on how to proceed with a 24-hour urine collection, where they get information about preparation for test, the best container, the volume necessary for testing, fluid intake and how to proceed if they need to subsequently collect a random urine sample. They were also asked if they would accept to repeat the collection if necessary and if they would warn the laboratory personal if they lost one or more micturition during the 24-hour period. After being compiled the frequencies of each response were calculated and expressed as a percentage basis. **Results:** Regarding the medical request, 25% of the patients stated that they did not know their physicians ordered a laboratory test that requires a 24-hour urine col-

lection. The laboratory was the primary source of information on the procedures to be followed for the appropriate collection of urine samples (50% of the cases). It is noteworthy that 19% of the patients reported not receiving any kind of professional information. Thirty percent of the patients were not able to correctly inform the collection procedures or the types of containers that could be used as an alternative to the ones provided by the laboratory. Concerning the importance of not losing any micturition during the collection period 35% of the patients were unaware of this matter and more than 50% of the subjects did not know what to do if the urine volume exceeded the containers delivered by the laboratory. It is remarkable that 10% of the patients stated that they would not inform the laboratory if they lost one or more micturitions. Another critical finding is the fact that more than 80% of patients said they had not been informed about the fluid intake during the collection period. When asked how to proceed in case they needed to collect a subsequent random urine sample, only 75% of the patients reported the correct procedure. **Conclusion:** This study allowed us to identify information gaps for the adequate 24-hour urine collection which will permit to elaborate an educational planning in order to improve this procedure aiming to reduce laboratory errors in the preanalytical phase

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Clinical significance of discrepant ELISA and IFA results for anti-PLA2R antibody testing

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Background: Recently, phospholipase A-2 receptor 1 (PLA2R) was identified as a major target antigen in the pathogenesis of idiopathic membranous nephropathy (MN). Detection and quantification of anti-PLA2R autoantibodies (aPLA2R) is critical for the diagnosis and management of MN as primary forms require immunosuppressive treatments while secondary forms may not. Indirect immunofluorescence (IFA) and enzyme-linked immunosorbent assay (ELISA) kits for aPLA2R measurement in serum/plasma are commercially available and quite sensitive and specific for initial diagnosis but performance characteristics of these kits are not established for patients receiving therapy. Nevertheless many aPLA2R tests are ordered for monitoring response to therapy. While our initial observations suggest that the ELISA titers typically fall with effective treatment, the IFA often remains positive, at least initially, causing confusion. This study investigated the clinical significance of discrepant IFA and ELISA results. The aim of our study was to determine the clinical utility of concurrent IFA and ELISA aPLA2R testing. **Methods:** This study was reviewed and approved by the Mayo Clinic Institutional Review Board. Results (n=538) from Mayo Clinic patients with clinically ordered aPLA2R testing from July 2015 through December 2016 were reviewed. IFA and ELISA testing were performed per manufacturer's instructions on all orders. IFA reactions were recorded as positive, negative or indeterminate (if background staining obscured technicians' judgement); indeterminate results were considered negative. ELISA results ≥14 RU/mL were considered positive as per the package insert. Patients with at least one set of conflicting assay results) were identified and their clinical record reviewed. **Results:** Observed agreement between the IFA and ELISA was 92% with discordant results noted in 24 unique patients. ELISA was positive in 13 samples with a negative IFA result and IFA was positive in 28 samples with a negative ELISA. Of the 41 discrepant results, 37 (90%) were from patients with biopsy-proven MN, two (5%) were duplicate orders from a patient with membranous lupus nephritis (further repeat testing yielded negative results), one (2.5%) was from an IgA nephropathy patient, and one had no biopsy performed. In biopsy-proven MN cases, 33/37 (89%) of dissimilar results came from patients (n=19 unique) who were currently receiving therapy (n=27) or in clinical remission (n=6). One instance of an indeterminate IFA and positive ELISA was observed. ROC analysis of the entire cohort demonstrated excellent ability of the ELISA to predict a positive IFA result (AUC = 0.97). SE+SP was maximized (0.94 and 0.92, respectively) at an ELISA result of 6 RU/mL. **Conclusion:** Good agreement was observed between assays, with the majority of incongruent results occurring in patients with biopsy-proven MN. In the vast majority of discrepant cases (80%) the patient was receiving immunosuppressive therapy, and the trends in the antibody titer by ELISA might be most revealing. It is unlikely a positive IFA result adds much value in this circumstance. The clinical significance of a positive IFA and negative or equivocal ELSA in newly diagnosed patients, or whether a positive IFA might predict early relapse in a treated patient when the ELISA result is equivocal, remains to be seen.

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Comparison of Multiple Analytical Approaches for Determining Reference Intervals

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BACKGROUND: Reference intervals are essential interpretative information for clinical assays. CLSI guidelines recommend both a non-parametric method as well as statistically more complex procedures such as bootstrap-based, parametric or specific robust methods for establishing reference intervals dependent on a laboratory's reference population sample size and access to sophisticated statistics. The non-parametric method which ranks values and drops the top and bottom 2.5th percentiles to determine the central 95% is widely used in clinical laboratories and appears to be ideally suited for *a priori* approaches that utilize "healthy" populations with at least 120 reference subjects. The goal of this study was to compare different statistical methodologies for determining reference intervals of 29 chemistry analytes from patient data extracted from our electronic health record system. **METHODS:** IRB approval was obtained for three datasets consisting of outpatient visits to our institution between 2012 and 2017 as follows: 1) All unique encounters with a strict qualifying encounter diagnosis code for general adult examination without abnormal findings, 2) All unique encounters with a more expansive qualifying encounter diagnosis codes for screening purposes, and 3) all encounters with at least 3 results per analyte per patient which were then averaged. Statistical methods used were EP Evaluator non-parametric analysis with outlier detection, Python non-parametric analysis, Prism 7 parametric analysis with outlier detection, R parametric analysis based on the Hoffman method, and R parametric analysis based on the maximum likelihood method from the mixtools package. Each statistical method was compared to the proposed reference interval in package inserts, an external reference laboratory, and our current reference interval for accuracy. Percent bias from the current reference interval was calculated. The number of samples was analyte dependent but ranged from 128-23713. **RESULTS:** The Hoffman and maximum likelihood methods had the tightest clustering among the five methods and most closely recapitulated the ranges in the package insert and external reference laboratory across all three datasets. The averaged dataset had greater effect on clustering in non-parametric methods, likely because it contained more unhealthy individuals. In comparison, the parametric Hoffman and maximum likelihood methods were less likely to be influenced by outlier values. Bias analysis for lower limit revealed <±25% bias for 86% of the analytes using non-parametric methods as compared to <±50% bias for 85% using parametric methods. Bias analysis for the upper limit showed >±50% bias for all analytes using non-parametric methods as compared to <±50% bias for 97% of analytes using parametric methods. **CONCLUSION:** For *a posteriori* approaches, which rely on populations likely containing unhealthy individuals, a non-parametric approach does not distinguish between the sub-populations while Hoffman and maximum likelihood methods do. Unlike the Hoffman method, the maximum likelihood method does not depend on subjective visual discrimination and thus is more advantageous.

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Calprotectin Antibodies with Different Binding Specificities Can Be Used as Tools to Detect Multiple Calprotectin Forms

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Background: Calprotectin is a calcium-binding protein secreted by neutrophils at a site of inflammation. In clinical diagnostics, analysis of fecal calprotectin is commonly used to diagnose intestinal inflammations, especially inflammatory bowel disease (IBD). Calprotectin is a complex protein composed of two subunits, S100A8 and S100A9. The two subunits associate together as heterodimers, and the dimers often pair to form tetramers. This multimerization tendency has been suggested to be a major reason for the observed substantial differences between the numerical values reported across commercial calprotectin assays. Depending on the specificity of the antibodies used in each assay, different multimer forms may be detected in patients' samples. **Methods:** We have developed five mouse monoclonal antibodies against human calprotectin. The binding specificities of these antibodies, designated as Anti-h Calprotectin 3403, 3404, 3405, 3406, or 3407, were studied in fluorescence-based immunoassays (FIA) with purified calprotectin subunits S100A8 and S100A9, as well as with the S100A8/A9 heterocomplex (i.e. calprotectin). Purified recombinant antigens were coated onto microtiter plate wells, 50 ng/well. A dilution series of antibodies was added into the wells at concentrations ranging from 0.031 to 10 ng/mL. The antibodies bound to the antigens were detected using an europium-labeled rabbit anti-mouse IgG antibody. Selected antibody pairs were used in sandwich fluoroimmunoassays to study the effects of antibody specificity differences on calprotectin assay results. The amount

of capture antibody was 150 ng/well. A dilution series of S100A8/A9 complex was added into the wells, at concentrations ranging from 0.15 to 1,000 ng/mL. Biotin-conjugated antibodies and Eu-labeled streptavidin were used to detect the bound antigens. A widely studied calprotectin antibody 27E10 was included in the study as a reference. **Results:** Direct FIA results with purified recombinant S100A8, S100A9, and S100A8/A9 complex proteins indicated that the antibodies were clustered in three groups: 1) antibody 3403 which bound to subunit S100A9, 2) antibodies 3404-3406 which recognized the subunit S100A8, and 3) antibody 3407 which did not bind to either of the isolated calprotectin subunits. All antibodies recognized the S100A8/A9 complex. Sandwich FIA results varied significantly between different antibody pairs. Antibody 3407 yielded similar results as the reference antibody 27E10 when used as a pair with itself. Significantly higher signal-to-noise ratios were obtained across the concentration range tested when 3407 was combined with an antibody recognizing a specific subunit, such as antibody 3406. **Conclusion:** These results demonstrate that calprotectin antibodies have different specificities towards calprotectin subunits and that the choice of antibodies for a calprotectin immunoassay have a significant effect on the detection results. Scientific interest towards the use of calprotectin as a biomarker for several diseases linked to subclinical or clinical inflammation has increased substantially during the past years. The correlation of S100A8/A9 tetramer, dimer, or monomer levels with the patients' disease status remains to be elucidated. Antibodies described in this study can be used as a tool to develop new diagnostic assays for distinct calprotectin forms.

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Biotin interference in 21 immunoassays performed on the Vitros5600

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Background: Biotin (vitamin B7) is a carboxylase co-factor involved in amino acid, fatty acid, and glucose metabolism. Select immunoassays are susceptible to biotin interference at supraphysiological concentrations. Cases involving administration of biotin supplements that exceed the recommended daily intake have been reported by physicians at our institution with concern for potential biotin interference with patient testing. This study aimed to characterize biotin interference with 21 immunoassays performed using the Vitros5600 (Ortho Clinical Diagnostics, Raritan, NJ), and evaluate the effectiveness of streptavidin-microparticle-mediated biotin depletion in serum samples. **Methods:** Increasing concentrations of biotin (0, 50, 100, 500 ng/mL; Sigma Aldrich, St. Louis, MO) were added into serum containing low or high analyte concentrations followed by testing for 21 analytes via immunoassays on the Vitros5600. Relative bias was calculated (%bias=([biotin-treated analyte]-[untreated analyte])/[untreated analyte]). Biotin depletion was evaluated by comparing serum with and without biotin (1000 ng/mL) and streptavidin-microparticle pretreatment (Thermo Fisher Scientific, Waltham, MA). Analyte recovery was calculated (%recovery=[biotin+streptavidin-microparticle treated analyte]/(untreated analyte]). **Results:** Biotin (50 ng/mL) caused negative biases in 15 immunometric assays, ranging from -2.9% for ferritin to -94.7% for cTnI. Biotin (50 ng/mL) elicited positive biases in 6 competitive immunoassays, ranging from 0% for cortisol to 2,270% for estradiol. Recovery of expected values following biotin and streptavidin-microparticle pretreatment was 99% to 115% for all immunoassays. **Conclusion:** The magnitude of analytical bias due to biotin interference is highly variable among immunoassays. Immunometric assays exhibit negative proportional biases that are dependent on biotin concentration, although appear independent of analyte concentration. Competitive immunoassays exhibit positive biases that are dependent on both biotin and analyte concentrations. Biotin depletion using streptavidin-microparticles is an effective method to recover expected analyte concentrations. Understanding patterns of biotin interference and implementing biotin depletion studies can aid in the investigation of biotin interference in clinical practice.

Test	Method	Analyte concentration	% Bias with added biotin (ng/mL)			
			0	50	100	500
PTH	IM	50.9 pg/mL	0	-73.9	-84.8	-91.6
		2115 pg/mL	0	-67.7	-80.1	-91.5
cTnI	IM	0.161 ng/mL	0	< AMR	< AMR	< AMR
		8.75 ng/mL	0	-94.7	-97.1	-99.1
TSH	IM	2.45 mIU/mL	0	-93.1	-95.9	-98.0
		59.36 mIU/mL	0	-89.4	-93.7	-96.6
Cortisol	Comp IA	1.33 mcg/mL	0	8.3	547	> AMR
		5.56 mcg/mL	0	0	414	> AMR
Estradiol	Comp IA	21.3 pg/mL	0	2270	8676	> AMR
		225.3 pg/mL	0	960	> AMR	> AMR
Testosterone	Comp IA	7.79 mg/dL	0	746	2467	14021
		86 mg/dL	0	269	745	> AMR

AMR, analytical measuring range; Comp IA, competitive immunoassay; IM, immunometric assay. Data not shown for AFP, bhCG, CA 19-9, CA 125, CEA, CK-MB, ferritin, folate, FSH, LH, NT-pBNP, progesterone, prolactin, PSA, or vitamin B12.

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Remediation of IgG4 Cross Reaction from The Binding Site Optilite® IgG1 and IgG2 Assays

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Introduction

Historically, IgG subclass (IgGSC) measurements to aid the diagnosis of primary immunodeficiency and hypergammaglobulinemia were performed using radial immunodiffusion (RID). This technology was superseded by fully automated and quantitative nephelometric and turbidimetric assays. The use of mass spectrometry for the identification of immunoglobulin subclasses has also been investigated. Whilst good agreement between the immunoassay techniques and mass spectrometry has been observed, discordance between the methods may exist for IgG1 and IgG2 measurements in patients with IgG4-related disorders (IgG4-RD). Elevated IgG4 levels are present in IgG4-RD, which has an estimated prevalence of only 0.28–1.08/100,000 of the adult population. As the clinical presentation and age of onset for immunodeficiency and IgG4-RD are distinct, the clinical utility of the IgGSC assays will be unaffected. However, over-estimation in the presence of an interfering substance is an undesirable characteristic. Here we assess IgG4 cross reaction in the Optilite IgG1 and IgG2 assays and describe its removal from the assay antisera.

Method

Polyclonal IgG4 was purified from a pool of human sera from healthy adult donors using standard chromatography techniques. Interference was established following CLSI guideline EP07-A2 at the lower limit of adult reference ranges for IgG1 (3.8g/L) and IgG2 (2.5g/L) using IgGSC assays (The Binding Site Group Ltd., UK) performed on the Optilite® (The Binding Site Group Ltd., UK). Base pools were spiked with either saline or an equivalent volume of purified IgG4 to give an IgG4 concentration of either 2g/L or 4g/L. Absorption chromatography was used to remove cross reacting antibodies to IgG4 and the antisera was then concentrated. Interference was reassessed as above, reference ranges were validated (n=51) and a comparison was made between the absorbed and unabsorbed assays using a panel of processed samples (IgG1 n=22; IgG2 n=23) spanning the analytical measuring range. Linear regression and Altman-Bland were performed using Analyse-it®.

Results

Prior to IgG4 adsorption, the IgG1 base pool sample spiked with 2g/L of purified IgG4 showed a 21% increase in reported concentration; when spiked with 4g/L, an increase of 38% was seen. This resolved to an increase of just 4% when spiked with either 2g/L or 4g/L IgG4 after adsorption. For the IgG2 assay, a 57% and 98% increase in result was observed when spiked with 2g/L and 4g/L IgG4, respectively. This resolved to just 3% (2g/L) and 10% (4g/L) post adsorption. IgG1 and IgG2 reference ranges were validated with a bias of -2.5% and 1.9% respectively. Processed panel sample results using absorbed and unabsorbed assays compared well (IgG1: $Y=1.016x-232.17$, $R^2=0.9919$; IgG2: $Y=1.0341x-123.34$, $R^2=0.996$).

Conclusions

IgG4-RD is a rare group of disorders and the over-production of IgG4 represents a new interference consideration for in-vitro diagnostic manufacturers. Purified polyclonal IgG4 can be used to assess antibody specificity following CLSI guideline

EP07-A2 and absorption chromatography used to remove undesirable IgG4 recognition; both will be incorporated into future Optilite subclass assays. Removal of IgG4 cross reacting antibodies did not alter the normal reference range for IgG1 and IgG2 nor did it negatively impact the standard QC panel.

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Evaluation of L-Index Interference Limits on Roche cobas c502 and c702 Immunoturbidimetric Assays using Endogenously Lipemic Specimens

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Background: Specimen lipemia is a primary concern with turbidimetric and nephelometric assays due the potential interference caused by light scattering or absorption. Lipemic index (L-index) and/or triglyceride (TRIG) concentration thresholds are frequently determined by manufacturers and provided in corresponding package inserts. Lipemic interference studies are typically conducted using soy-based lipid emulsions (e.g. Intralipid), which simulate endogenous lipemia. Intralipid, however, may not fully represent interference caused by endogenous lipemia due to the complexity and diversity of human lipoproteins. The purpose of this study was to evaluate lipemic interference thresholds across eleven FDA-cleared assays using patient specimens with varying degrees of endogenous lipemia pre- and post-ultracentrifugation (UC). **Methods**: Residual human serum specimens (n = 42) were obtained from frozen storage (-20°C) and de-identified according to an IRB-approved protocol. Specimens were retrieved based on prior L-index and/or TRIG measurements. Samples were tested untreated as well as after treatment by UC (AirFuge; Beckman Coulter; Brea, CA). Baseline and post treatment testing was conducted on two Roche instruments - cobas c502 [α1-antitrypsin (AAT), complement C3c (C3), C-reactive protein (CRP), haptoglobin (HAPTO), soluble transferrin receptor (STFR)] and cobas c702 [β2-microglobulin (B2M), complement C4 (C4), ceruloplasmin (CERU), high sensitive C-reactive protein (hsCRP), prealbumin (PREA), and transferrin (TRSF)]. Serum indices and TRIG concentrations were also measured pre- and post-UC. Assay results which fell outside the analytical measurement range were excluded due to the confounding effect of manual or auto-dilution on baseline lipemia. Percent difference of assay results pre- and post-UC - calculated as [((post-pre)/pre)*100)] - were determined for each result pair and used to establish L-index interference thresholds (≥10%) using non-linear regression in SigmaPlot 13 (Systat; San Jose, CA). **Results**: Specimens had serum indices pre-UC spanning the non-lipemic to lipemic range (L-indices, 1-1769) with minimal hemolysis (H-index ≤85) or icterus (I-index ≤2). UC did not adversely impact results in non-lipemic specimens (n=11, L-index ≤50). UC was effective at clearing lipemia (post-UC L-index: 16 ± 8), although persistence of residual TRIG without corresponding L-index elevation was often observed. Increasing lipemia caused a negative interference in AAT, HAPTO, TRSF, and PREA assays, a positive interference in CRP, CERU, and hsCRP assays, and negligible effect in B2M, C3, C4, and STFR assays across the L-index range evaluated. Several assays showed L-index thresholds that were below previously defined limits from the package inserts [new (prior)]: AAT 300 (500); CRP 230 (1000); hsCRP 300 (600); HAPTO 440 (600); TRSF 225 (500). **Conclusions**: This study provides an analysis of L-index thresholds for eleven immunoturbidimetric assays. Due to the variety of human lipoproteins, limits defined using endogenously lipemic patient specimens may be different from those derived from spiking studies using Intralipid.

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Correlation of the Hemolysis Index on the Vitros® 5600 Analyzer with HemoCue® Photometer Plasma Hemoglobin Values

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Background: Multiple analytes measured in the clinical laboratory are affected by hemolysis. Most automated chemistry analyzers provide a hemolysis index (H index) on each specimen that can be used to monitor the degree of hemolysis, but this does not directly measure the concentration of hemoglobin present. It is the responsibility of each laboratory to determine the H index to be used in the decision to either report a result, report the result with an interpretive comment, or cancel the test due to hemoglobin interference. There are some instances, such as the monitoring of in vivo hemolysis in patients on extracorporeal membrane oxygenation (ECMO) when the direct measurement of hemoglobin concentration in plasma is clinically indicated. In our laboratory, in ECMO patients, plasma hemoglobin is measured by the HemoCue photometer. We ran into a situation when reagents/cuvettes were unavailable for the HemoCue. We investigated the possibility if the H index could be used to give an approximation of the plasma hemoglobin concentration. In the present study, we compared the H index reading given by the Vitros 5600 to the measured hemoglobin in plasma specimens by the HemoCue photometer. We also investigated the use of a regression equation to calculate plasma hemoglobin concentrations using the H value from the Vitros. **Methods:** 29 scavenged specimens were spiked with a washed red cell hemolysate. They then had plasma hemoglobin measured by the HemoCue photometer and the H index measured on two Vitros 5600 analyzers in use in the clinical laboratory. Based on hemoglobin values from the HemoCue, the Vitros H index values were divided into the following groups: no hemolysis; slight, moderate, and gross hemolysis; and reject. A regression equation was derived that could be used to calculate approximate plasma hemoglobin values based on the Vitros H index value. **Results:** H index values from the Vitros were divided into the following categories after comparison with HemoCue hemoglobin measurements: 1-100 H index, no hemolysis; 100-200 slight hemolysis; 200-300 moderate hemolysis; 300-500 gross hemolysis; >500 reject. The following regression equation was determined that could approximate hemoglobin concentration for specimens with slight hemolysis (y=0.73X + 10, where y= the H index and X= hemoglobin in mg/dL). **Conclusion:** After comparing the Vitros H index results with the HemoCue hemoglobin measurement, we were able to more accurately designate specimens into varying categories of hemolysis. This allows a more precise determination of when to cancel tests whose results are affected by hemolysis. The regression equation will allow plasma hemoglobin values to be reported without a disruption in clinical care if a reagent shortage would occur.

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Ensuring Assay Consistency through the Prediction of Lot to Lot Variation, Using Regression Analysis- Derived Theoretical Concentrations for Ferritin

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Background: Ensuring assay consistency over time is an important part of the laboratory's quality program. Variation between reagent lots over time can cause changes in the proportion of abnormal results. Our current lab practice is to assess each new lot of reagent by performing sample comparisons between the current and new lot and use Passing-Bablok regression analysis to compare results. Long-term impact of lot-to-lot variation at relevant medical decision points is further assessed by applying the regression analysis equation to a theoretical low, normal, and high test value and monitoring changes in the theoretical value over time. **Objective:** The aim of this study was to use Ferritin as a model analyte to determine whether lot-to-lot variation observed using sample comparisons and regression analysis-derived theoretical values is predictive of variation in reported patient results. **Methods:** Ferritin was measured using the DxI 800 (Beckman Coulter, Inc., Brea, CA). Reported patient results (n=188,087) were captured from the laboratory information system from 08/2011-05/2017. Reagent lots were compared using residual serum samples (n=20). Passing-Bablok regression analysis was performed and equations were applied to theoretical values at clinical decision limits (10, 336, 500 mcg/L) to monitor variation. Median patient results, interquartile range (IQR), and percent above and below reference intervals were calculated for reagent lots considered either stable or changing. The stable period had slope evenly distributed around 1.0 over at least three lots. The changing period had slope >or<1.0 over multiple consecutive lots. Comparisons between the median patient values in the stable and changing time periods were performed using Wilcoxon Rank Sums test for statistical significance. Comparison of the percentage of samples flagged low or high in the stable and changing data sets were calculated using a pooled estimate 2-sample test of proportions. Statistical calculations were performed using JMP Pro version 13.0.0 (SAS Institute Inc., Cary, NC) where $p<0.05$ was statistically significant. **Results:** The stable period consisted of 3 reagent lots (08/2011–12/2012) having a mean(range) slope=1.0(0.97-1.02). The changing period consisted of 8 reagent lots (01/2013-05/2017) having mean(range) slope=1.05(1.01-1.11). The average(maximum) difference in the 10 mcg/L theoretical concentration was stable=1.8(3.2) and changing=-7.5(-20.7). The average(maximum) percent difference in the 336 mcg/L theoretical concentration was stable=4.8(5.2) and changing=19.4(25.6). The average(maximum) percent difference in the 500 mcg/L theoretical concentration was stable=3.7(5.0) and changing=19.4(25.6). The median patient values(IQR) were 47(20-140) for the stable period and 47(20-133) for the changing period ($p=0.12$). The percent(SD) of samples flagging low was 17.3(1.7) during the stable period and 16.5(0.9) during the changing period ($p<0.0002$), and percent of samples flagging high was 12.7(1.4) for the stable period and 12.2(0.7) for the changing period ($p<0.0093$). Though the percent of samples flagging low and high were statistically significant, likely due to large sample size,

they were deemed clinically insignificant due to overlap of IQR of the data sets. **Conclusion:** These data suggest that use of theoretical values derived from lot checkout data falsely alerted the lab of assay drift. Patient medians in conjunction with % of results flagging low or high better reflect assay stability over time.

A-288

HbA1c platforms are variably affected by increasing lipemia

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Background: Hemoglobin A1c (HbA1c) testing is central in the diagnosis and monitoring of diabetes mellitus. Several analytical approaches for measuring HbA1c are available including those routinely used in clinical laboratories: high performance liquid chromatography (HPLC), capillary electrophoresis (CE), enzymatic, and immunoassay (IA). Considerable attention has been paid to interference from hemoglobin variants in HbA1c methods, with less investigation of matrix-related interferences such as lipemia. Interference from clinical lipemia in HbA1c measurements is particularly relevant as non-fasting specimens are accepted and dyslipidemia is common in diabetic patients. **Objectives:** (1) To investigate the concentration of Intralipid®-sourced triglycerides that may cause significant interference on four platforms representing common analytical methods for HbA1c. (2) To assess the performance of nine routine HbA1c platforms using clinically lipemic specimens. **Methods:** Four specimens with 7.1 to 7.5% HbA1c were aliquoted and spiked with saline and/or Intralipid to generate triglyceride levels of 0, 5, and 20 g/L. Specimens were measured on the Bio-Rad VARIANT™ II (VII) TURBO 2.0 (HPLC), Sebia CAPILLARYS™ Hb A1c (CE), Abbott ARCHITECT™ Hemoglobin A1c (enzymatic), and Roche COBAS® c501 Tina-quant® HbA1c Gen. 3 (IA). Remnants of whole blood specimens (n=40) visually identified as lipemic and ranging from 3.8 to 14.5% HbA1c were tested on nine HbA1c platforms, including the four listed above and the Bio-Rad D-100™ HbA1c (HPLC), Bio-Rad VII HbA2/HbA1c Dual Program (HPLC), Beckman Coulter AU® HbA1c (IA), Ortho VITROS® HbA1c (IA), and Siemens Dimension Vista® HbA1c (IA). Paired plasma specimens were assayed for triglycerides on the ARCHITECT. Data were processed in Microsoft® Excel. A significant difference was defined as >6% change from baseline (0 g/L Intralipid) or >10% change from the average value reported by platforms with claimed resistance to clinical lipemia interference (VII TURBO and HbA2/HbA1c). **Results:** The VII TURBO and CAPILLARYS reported HbA1c values without significant change from baseline in the presence of up to 20 g/L Intralipid-sourced triglycerides. However, the ARCHITECT and COBAS HbA1c values were negatively biased by 10% and 25% at 5 g/L and 20 g/L triglycerides, respectively. For clinically lipemic samples, the all-methods coefficient of variation correlated with increasing concentration of triglycerides (R^2=0.59). The Bio-Rad D-100, CAPILLARYS and Dimension Vista HbA1c results did not show significant bias up to 65 mmol/L (57 g/L) triglycerides for 100%, 95% and 81% of specimens, respectively. The ARCHITECT and VITROS HbA1c values were significantly depressed above a 10 mmol/L triglyceride threshold (-16% and -19% average bias, respectively), with bias in proportion to the degree of lipemia. The AU and COBAS also showed significant bias above 10 mmol/L triglycerides (-12% for both). **Conclusion:** This study revealed that most immunoassays and the enzymatic method for HbA1c are susceptible to negative interference from elevated triglycerides, while HPLC and CE methods are resistant. To avoid reporting falsely low HbA1c measurements, laboratories should consider evaluating their assay performance for significant interference from clinical lipemia. Although further investigations are needed, our data suggest that a serum triglyceride threshold of ~10 mmol/L may warrant a cautionary note when reporting HbA1c or reflexive testing to a lipemia-resistant platform.

A-289

Interference of acetone with the alkaline-picrate method for blood creatinine measurement on the Abbott Architect

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Background: Acetone is known to cause a positive interference when measuring creatinine using the alkaline-picrate method (Jaffe reaction). However, this phenomenon has not been reported for the assay performed on the Abbott Architect, nor is it recorded as a known interference in the package insert for this assay. This interference was brought to our attention when a 33-year old male presented to the emergency department at our institution following consumption of rubbing alcohol (isopropanol). Isopropanol is metabolized to acetone in the body, resulting in an elevated creatinine level when measured using this method. This can lead to the inaccurate diagnosis of acute kidney injury and, subsequently, inappropriate treatment. A member of the patient's care team was suspicious of the elevated creatinine and contacted the laboratory. We were able to test the patient's blood by an alternate method, revealing a creatinine concentration within normal limits. The objective of this study is to investigate the effect of isopropanol and acetone on the measurement of creatinine using the alkaline-picrate based method on the Abbott Architect *c* system. **Methods:** We performed interference studies using two levels of BioRad Multiqual quality control (QC) reagents (mean creatinine concentrations of levels 1 and 3 = 0.65 mg/dL and 6.16 mg/dL, respectively) supplemented with either isopropanol or acetone to a target final concentration of 0 - 400 mg/dL. Samples were divided and assayed for creatinine on the Abbott Architect *c* system, and also underwent gas chromatography with flame ionization detection for the quantification of volatile compounds (i.e. isopropanol and acetone). **Results:** The presence of isopropanol did not affect the measurement of creatinine however, acetone displayed a positive interference that increased with acetone concentration. The highest concentration of acetone tested (mean 330.3 mg/dL) resulted in a 0.18 mg/dL (27%) and a 0.20 mg/dL (3%) increase in creatinine concentration at level 1 and level 3 QC material, respectively. **Conclusion:** This study demonstrates that acetone can falsely elevate creatinine measurement performed on the Abbott Architect. This has important implications for patients following isopropanol ingestion, as spurious results may lead to unnecessary treatment of renal failure. In these patients, creatinine measurements determined using this platform should be interpreted with caution, and an alternate creatinine methodology should be considered.

A-290

Protein Gel Formation Caused the Interference of the Total Bilirubin Assay on the Roche Cobas 8000

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Objective: Monoclonal immunoglobulin paraproteins (M-protein) present in serum or plasma samples in patients with multiple myeloma, Waldenstrom's macroglobulinemia, plasmacytoma, amyloidosis, and monoclonal gammopathy of undetermined significance (MGUS) can interfere with a wide range of chemistry or immunochemistry tests. M-protein precipitation and aggregation under an assay condition are the most important causes of the interference. Irreproducible results or fluctuation of results upon repeat is the characteristic pattern of M-protein interference caused by protein precipitation. In order to add to our understanding of the mechanisms of M-protein interference, we present an unusual case of interference due to protein gel formation on the total bilirubin assay. **Case Presentation:** A patient with recurrent multiple myeloma characterized by multiple high-risk cytogenetic and molecular abnormalities was seen at our institution and an elevated total bilirubin result of 7.2 mg/dL (reference interval (RI), 0.0 - 1.2 mg/dL) was obtained on the Roche Cobas 8000 automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Other abnormal laboratory findings included elevated IgG of 10,400 mg/dL, lambda free light chains of 199.5 mg/dL with a kappa/lambda ratio < 0.01, beta-2 microglobulin of 6.2 mg/L (RI, 1.0-2.1), and total protein of 13.8 g/dL (RI, 6.1-8.2). Given the lack of clinical indications for liver disease, the clinical team questioned our total bilirubin result. **Method and Results:** Our investigation included three experiments: repeat testing in triplicate to conform the result, serial dilution to determine the presence of any interference, and mixing study to observe the reaction. The repeat results were 7.3 mg/dL, 7.3 mg/dL, and 7.2 mg/dL, indicative of consistent readings that did not fluctuate. The dilution experiment obtained total bilirubin results of 0.9 mg/dL, 0.3 mg/dL, and 0.1 mg/dL on two, three, and five times diluted samples, respectively, indicating poor recovery and lack of parallelism. Following the same sample to reagent ratio of 1:72 as that performed on the Cobas 702 instrument and mixing 20 µL of the sample with 1200 µL of the R1 reagent (containing 26 mmol/L phosphate, detergent, and solubilizers, pH 1.0) and 240 µL of the R2 reagent (containing 3,5-dichlorophenyl diazonium) of the Cobas total bilirubin (Gen.3) assay in a test tube, we observed the formation of a soft opaque gel. When the ratio of sample to R1 reagent was increased to 1:1, the entire solution turned into a single transparent gel in the tube. **Discussion:** Assay conditions, especially pH and ionic strength, can induce protein conformational change. The previous studies explained that the precipitates or aggregates of M-protein caused a fluctuating pattern of repeated results due to random movement of protein aggregates/precipitates altering optical measurements. This case illustrated a non-fluctuating pattern upon repeat. This particular M-protein cross linked and formed a gel when changing its conformation at low pH of the total bilirubin assay. The M-protein formed a stationary opaque gel which led to reproducible but incorrect absorbance measurement. This case study adds to our understanding of the mechanisms of M-protein interference with optical measurements of chemistry and immunochemistry assays.

A-291

Plasma Lactate Samples Collected in Fluoride/Oxalate Tubes Do Not Require Transport on Ice

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Background. Consensus guidelines for the management of sepsis and septic shock recommend baseline and follow-up lactate measurements (Surviving Sepsis Campaign, 2016). Lactate concentrations in whole blood samples can rapidly increase due to anaerobic glycolysis in erythrocytes. If testing is delayed, or if samples are not collected and transported appropriately, falsely elevated lactate results could lead to misdiagnosis and unnecessary treatment. Lactate production *in vitro* may be slowed by chilling the whole blood on ice and/or by collecting the blood in tubes containing glycolytic inhibitors, such as fluoride/oxalate tubes. Almost all diagnostic test manufacturers and most laboratories measuring plasma lactate currently recommend collection in gray fluoride/oxalate tubes on ice, transport on ice, and minimizing delays in testing. However, not all laboratories require that fluoride/oxalate lactate specimens be transported on ice or kept refrigerated. *The objective of this study was to determine stability of plasma lactate measurements in whole blood samples stored at room temperature after collection in fluoride/oxalate tubes.* **Methods.** Whole blood was collected in green-top lithium heparin (n=4) or gray-top sodium fluoride / potassium oxalate (n=42) BD Vacutainer® plastic tubes and sent to the laboratory on wet ice. The whole blood was resuspended, and de-identified aliquots were made. The primary tubes were centrifuged and tested immediately to obtain initial values for plasma lactate. After storage at room temperature or 4°C for various lengths of time, the whole blood aliquots were centrifuged to obtain plasma. Lactate measurements were made using either Beckman Coulter DxC800 or Roche Cobas automated methods. **Results.** Plasma lactate concentrations steadily increased in lithium heparin samples, as expected for whole blood at room temperature. Whole blood aliquots from fluoride/oxalate samples maintained plasma lactate concentrations within allowable error limits of ±0.4 mmol/L or 10%. Fluoride/oxalate samples had starting lactate concentrations ranging from 0.7 to 7.2 mmol/L. Samples stored for up to 3 days at room temperature (n= 29) had a mean increase of 0.1 mmol/L and maximum increase in 3 samples of 0.2 mmol/L. Samples stored for up to 11 days at 4°C (n = 13) had a mean increase of 0.2 mmol/L and maximum lactate increase in one sample of 0.5 mmol/L or +7% from an initial value of 7.2 mmol/L. **Conclusions.** It is widely believed that fluoride/oxalate tubes must be transported on ice and stored at refrigerated temperatures. While this is needed for glucose analysis, these data suggest that low temperature storage and transport are not necessary for lactate samples collected in fluoride/oxalate tubes. And if testing is delayed, the lactate may still be measured and reported from fluoride/oxalate tubes stored up to 3 days at room temperature. Removing the requirement for low temperature transport and storage may simplify and expedite testing for patients with sepsis.

A-292

Accuracy of prediction of ovulation by digital home ovulation tests

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Background: Home ovulation tests are a useful home diagnostic that enables women to time intercourse in order to maximise their chances of conception, if accurate. Ovulation tests rely on daily measurement of urinary lutenising hormone (LH) by lateral flow, with a surge in levels occurring approximately 1 day prior to ovulation. Digital tests offer the advantage of removing ambiguity of interpretation and employ optical signal recognition and algorithms to determine day of surge. This study aimed to examine the accuracy of currently available digital tests. **Methods:** This study focused on 3 main digital home ovulation tests found in US retailers during 2017. Well at Walgreens consisted of 7 test sticks and a reader which deactivated after all tests were conducted or following LH surge detection; testing instructions were to test daily from day 8 of the cycle. First Response had 20 tests and a reader that deactivated once LH surge had been detected or all tests conducted; instructions were to test daily from day 5 of the cycle. Clearblue Advanced digital ovulation test tracks estrone-3-glucuronide as well as LH in order to identify the days of high fertility that precede the LH surge. It consisted of 20 test sticks and a holder, which did not deactivate, but reset after surge detected or non-usage; day on which to start testing was dependent on cycle length (with a look-up table to identify the start day), and users should continue testing until surge is detected. Daily urine samples from 33 women, with day of ovulation determined by transvaginal ultrasonography and an LH surge detectable in urine (level ≥40mIU/ml, AutoDELFIA) were tested with 3 batches of each test, according to their instructions for use. Agreement of ovulation test result with true day of ovulation was determined. **Results:** Well at Walgreens detected ovulation to within 1 day in 46.9% of cycles (95% CI: 36.6-57.3), with no increase in detection when considering to within 2 days. Ovulation was missed either, because testing began after LH surge had occurred, or because all tests were used and holder deactivated before reaching the surge day. First Response detected ovulation to within 1 day in 54.5% of cycles (95% CI: 44.2-64.6), and 65.7% (95% CI: 55.4-74.9) within 2 days with surges usually missed due to reader not detecting the surge, despite testing on the surge day. Clearblue detected 94.9% (95% CI: 88.6-98.3) within 1 day and 98.0% (95% CI: 92.9-99.8) within 2 days. **Conclusion:** Although digital ovulation tests are much easier to read and so have the potential for high accuracy as demonstrated by the Clearblue test, other parameters can reduce accuracy. Of key importance are; having a testing strategy that helps ensure a test is conducted on the surge day and having an optical detection system and algorithm that correctly identifies surge.

A-293

Prevalence of Biotin Interference in Samples Received for Routine Thyroid Function Testing

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Background: The increased use of biotin supplementation, and the subsequent influence this can have on laboratory testing, has become a hot topic. On the Beckman DxI platform, Total T3 (TT3), Free T3 (fT3), and Free T4 (fT4) are subject to biotin interference, which can falsely elevate results. TSH and Total T4 (TT4) are unaffected. The purpose of this study was to first, use historical data to estimate the prevalence of biotin interference, assuming that retrospective results showing a pattern of normal or elevated TSH with concurrent elevated thyroid hormone concentrations were likely candidates for biotin interference. Second, we sought to develop a protocol to detect biotin interference in these assays and to validate the method using samples with the suggested result pattern. Combined, these data provide us with quality assessment criteria to prospectively detect and evaluate samples received for routine thyroid function testing before erroneous results are reported. **Methods**: Paired TSH, TT3, fT3, TT4, and fT4 results were extracted from the LIS over a 12-month period. Results were filtered to exclude low TSH results (<0.4 mIL/L) and high TT4 results (>10.8 mcg/dL). Pooled serum samples adulterated with biotin (biotin concentration range 0 - 1,500 ng/mL) were tested on the DxI to evaluate interference in the thyroid hormone assays. Pooled serum samples and samples adulterated with 500 ng/mL biotin were incubated with various concentrations of streptavidin coated magnetic beads to establish endogenous thyroid hormone concentration recovery. **Results**: During the 12-month period ending 12/31/2017, we performed 72,843 TSH tests. After excluding paired results with low TSH and high T4, the remaining 67,628 results were then analyzed to identify result patterns suggestive of biotin interference, including elevated TT3, fT3, and/or fT4 in samples with high/normal TSH and normal TT4. These data estimated that 4.6% of all samples submitted for routine thyroid function analysis may have erroneous results due to biotin interference. Interference in TT3, fT3, and fT4 assays was confirmed by spiking biotin into pooled serum samples at concentrations ranging from 50 -1,500 ng/mL. The addition of biotin elevated previously normal levels to levels above the upper limit of normal for T3, fT3, and T4 at all concentrations tested. An assay using streptavidin coated magnetic beads was developed that identified biotin interference by detecting >75% recovery of TT3, fT3, and fT4, with a <10% change in TT4. **Conclusions**: The thyroid function tests for Total T3, Free T3, and Free T4 can be falsely elevated on the DxI platform as a result of biotin interference. This study estimates the prevalence of biotin interference in historical results, and details the development of pre-selection criteria, a sample testing method, and an evaluation protocol to identify biotin interference in these assays.

Tuesday, July 31, 2018

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

Hematology/Coagulation

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Does Vitamin B12 behave as an acute phase reactant

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Backgound: High serum vitamin B12 (vitB12) levels are found in myeloproliferative diseases including chronic myeloid leukemia, polycythemia vera, and also infectious and autoimmun diseases. The purpose of this study to determine whether high serum vit B12 levels may be used as an acute phase reactant. For this aim the inflammatory markers leukocytes (WBC), absolute neutrophil count (ANC), and erythrocyte sedimentation rate were investigated in patients whose serum vitB12 levels were above 1000 pg / mL associated with high or normal serum C-reactive protein (CRP) levels. **Methods:** Children who 0-18 years old were included to the study. The patients were divided into two groups: Group 1 (n=32, 17 F/15 M) containing the patients with high vitB12 (>1000 pg/mL) and high CRP (>0,8 mg/dL) levels, and Group 2 (n=19, 9F/ 10M) containing the patients with high vitB12 (>1000 pg/mL) and normal CRP (0-0,8 mg/dL) levels. Serum VitB12 assay was performed by competitive-binding immunoenzymatic assay on DXI800 (Beckman Coulter, Inc, US) with an analytical sensitivity of <50 pg/mL. Serum CRP was measured by nephelometry (Beckman Coulter, Inc, US). WBC was counted by Beckman DXH800 (Beckman Coulter, Inc, US) Absolute neutrophil count (ANC) was calculated. Erythrocyte sedimentation rate was measured by kinetic method of Test 1-ALIFAX (Italy). **Results:** The mean age of group 1 and 2 were 9,47 ± 5,77 (0,82-18,1) and 6,19 ± 5,3 (0,42-17,5), respectively. The median levels of the parameters are shown in Table 1. Table 1:

Conclusion: Only the ESR was significantly different between the two groups. This is a 50-patient sampling study in patients underlying different disorders and/or infectious. Extended study with different cut offs is planned.

Table 1

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Prevalence of Factor V Leiden and Prothrombin mutation assessed by data from a large clinical laboratory in Sao Paulo

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Background: Deep venous thrombosis (DVT) has multifactorial causes and 5-10% morbidity rate if not treated. Activated Protein C (mainly due to Factor V Leiden) and G20210A Prothrombin mutation are amongst genetic risk factors. Factor V Leiden (FVL) and G20210A Prothrombin mutation are due to punctual mutations that cause amino acid exchanges. Activated FVL looses Activated Protein C proteolytic site and becomes resistant to this enzyme's action. This mutation increases 3-8 times DVT risk in heterozygous patients, and 80 times in homozygous patients. G20120A Prothrombin mutation increases blood stream Prothrombin levels, causing hypercoagulability, yielding 2-3 times DVT risk. Therefore genetic thrombophilia diagnostic tests are important to reveal DVT cause and predict risk-benefit of anticoagulants treatment. The present study was performed to evaluate FVL and G20210A Prothrombin mutation prevalence amongst patients from a Sao Paulo large diagnostic laboratory. **Methods and Casuistic**: Peripheral blood samples collected into EDTA, using Biopur Extra Mini Spin Kit®. FVL and G20210A Prothrombin mutations were verified through Real Time PCR and allelic discrimination performed with TaqMan® probes in a 7500 RT (Applied Biosystems®) thermocycler. The data bank with FVL and G20210A Prothrombin mutation exam results was analyzed between 2006 and 2017. **Results:** From a total of 13286 patients, 10503 (79.05%) were female. The prevalence found for both mutations was similar to previous studies. FV Leiden prevalence was higher between male patients, as found in previous studies as showed in the table 1. **Conclusion**: It is important to diagnose FVL and G20210A Prothrombin mutation to evaluate anticoagulants treatment and use of birth control pills risk-benefits, as well to evaluate recurrent abortion risk in patients with 3 or more losses as a prenatal public health policy, since live births are significantly lower in women with recurrent early and late abortions who have FVL allele and in women who have Prothrombin mutation.

Table 1: FV Leiden and G20210A Prothrombin mutation prevalence.

FV Leiden	G20210A Prothrombin mutation			
	Wild Type	Heterozygous	Homozigous	Total
Wild Type	12033 (95.82%)	511 (4.07%)	14 (0.11%)	12558 (100%)
Heterozygous	675 (94.54%)	38 (5.32%)	1 (0.14%)	714 (100%)
Homozigous	13 (92.86%)	1 (7.14%)	0 (0%)	14 (100%)
Total	12721 (95.7%)	550 (4.1%)	15 (0.1%)	13286 (100%)

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Sysmex BeyondCaresm Quality Monitor: Closing the Loop on Quality Control

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Background: Quality control for a clinical laboratory is designed to detect issues related to the analytical process with the aim to maximize the quality of the results. Most laboratory quality control systems use a Levy-Jennings chart, where data is easily visible and trends and other changes clearly identified. Sysmex uses this method of quality control for hematology and has now added an additional layer on top of the standard Levy-Jennings charts, introducing the BeyondCare℠ Quality Monitor (BCQM) quality control management program. The application takes the guesswork out of quality control, and has the potential to identify quality control failures with minimum false rejection, ensuring analyzer reliability while providing a consistent quality control process. **Methods:** BCQM uses multiple algorithms incorporating Six Sigma methodologies to interpret the quality control data from the analyzer. A dashboard provides at-a-glance analyzer status tabs with real-time quality control information, a benefit for laboratories with varying degrees of skill. An analyzer with a green status indicates that precision and accuracy have passed the necessary criteria. If the status is yellow, more information is needed before patient testing can occur. If laboratory involvement is needed, the application will provide guided troubleshooting prompts to help the technologist resolve any issues and notify Sysmex for service if necessary. A red status indicates there is a precision or accuracy issue and generates a service request followed by a phone call from Sysmex. **Results:** MultiCare recently participated in a pilot study of the BCQM on the Sysmex XN-9000. An analyzer at one of the MultiCare Health System hospitals had a mean corpuscular volume (MCV) low bias that resulted in a standard deviation index (SDI) of -2.3 based on the BCQM criteria. A SDI result greater than ± 2.0 requires follow-up by the technologist reviewing the quality control data. The difficulty is that the daily quality control data was acceptable and within the normal ± 2SD non-BCQM criteria, so no apparent issue seen. This issue would not have been found until a monthly quality control review, possibly weeks later. BCQM caught this issue immediately and had service dispatched. The analyzer was calibrated and values went back to the mean compared to other analyzers. This issue is important because middleware rules dictate that the MCV will not autofile into the patient's chart with an MCV difference of ± 3.0 from the previous sample. The strong negative bias from this analyzer would cause many samples that would normally autofile be held up in the middleware, requiring action from a technologist. This increases turnaround time for patients, including emergency room patients. This unnecessary delay impacts throughput of these patients, which is critical for a high volume area. **Conclusion:** BCQM closes the loop on the quality control process for hematology. The complex algorithms are married to a simplified process that elevates the degree of quality while encompassing ease of use. This superior quality control model is invalu-

able to laboratories because major issues can be found and corrected quickly before any impact is seen in patient results.

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System performance evaluation of the cobas t 711 and cobas t 511 analysers

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Background: The fully automated **cobas t** 711 and **cobas t** 511 coagulation analysers perform qualitative and quantitative in-vitro coagulation analyses to aid the diagnosis of coagulation abnormalities and monitor anti-coagulation therapy. The objective of this validation study was to evaluate the functionality, reliability and analytical performance of the **cobas t** 711/511 analysers in a clinical laboratory setting under routine-like conditions at three European sites. **Methods:** Anonymised human citrated plasma samples were evaluated on the **cobas t** 711/511 analysers using a variety of coagulation tests. Functionality and analytical performance were monitored via daily quality control (QC) runs assessed twice daily. Intermediate precision was assessed over 21 days using control samples according to Clinical and Laboratory Standards Institute EP05-A3 guidelines. Routine Simulation Series (RSS) were used to confirm absence of random errors when running the analysers under routine-like conditions. Routine workloads of the laboratory were replicated and re-processed on the **cobas t** 711/511 analysers with the aim of verifying the performance by re-measuring all left-over specimens from the respective routine run. **Results:** All samples tested showed a stable QC performance over ~12 weeks. Of 4216 results, 19 were outside the ±2 SD (±10%) range due to reagent (or control material) related issues. Out of range results were repeated according to the QC re-run rules; all were resolved. No QC recovery issues due to reagent/instrument malfunction. All assays passed acceptance criteria for repeatability, intermediate and total precision of the respective assay (**Table**). For RSS, good comparability between runs was shown when testing single samples under random mode conditions (**Table**), no system malfunctions observed. **Conclusion:** The performance of the **cobas t** 711/511 analysers was excellent and the analysers are suitable for the accurate and reliable measurement of coagulation in routine clinical practice.

	aPTT	aPTT Lupus	aPTT Screen	D-Dimer[a]	Fibrinogen	PT-derived fibrinogen	PT Owren	PT	Thrombin time[a]
CV% day-to-day									
cobas t 711[b] / cobas t 511[c]	0.0-0.5 / 0.2-0.3	0.1-0.7 / 0.4-0.6	0.0-0.6 / 0.0-0.3	0.0-0.0; 0.0-0.0 / 0.6; 1	0.0-1.5 / 0.0-0.1	0.0 / 0.8	0.0-0.9 / 0.6-1.3	0.0-0.6 / 0.0-0.4	0.6-0.9; 0.0-1.2 / 0.9; 0.7
CV% within run									
cobas t 711[b] / cobas t 511[c]	0.2-0.4 / 0.3-0.4	0.3-0.6 / 0.4-0.5	0.3-0.9 / 0.6-1.0	1.4-1.5; 0.6-1.5 / 1.5-2.7; 1.0-2.0	1.2-3.0 / 1.7-2.6	1.5-2.5 / 2.1	0.4-1.3 / 0.7-0.9	0.3-1.1 / 0.3-0.5	1.0-2.0; 1.1-2.3 /1.1; 1.4
CV% total (within laboratory									
cobas t 711[b] / cobas t 511[c]	0.4-0.7 / 0.4-0.6	0.6-1.4 / 0.8-1.3	0.5-1.3 / 1.0-1.3	1.5-2.7; 1.0-2.0 / 2.4; 2	1.8-3.6 / 2.0-3.2	2.3-2.9 / 2.2	1.0-2.2 / 1.8-2.2	0.5-1.8 / 0.6-1.8	1.5-2.1; 1.9-2.5 / 1.6; 1.8
Correlation (Pearson's r)									
cobas t 711[b] / cobas t 511[c]	0.992-1.000 / 0.98	0.997-1.000 / 0.998	0.998-0.999 / 0.999	1 / 1	0.994-0.999 / -	0.995-0.999 / 0.998	0.999-1.000 / 1.000	1.000-1.000 / 1.000	0.967-0.991 / 0.952
Slope (Passing-Bablok)									
cobas t 711[b] / cobas t 511[c]	0.951-0.987 / 0.937	0.950-1.002 / 0.98	0.985-1.005 / 1	0.992-1.000 / 1.002	1.006-1.032 / -	0.987-1.000 / 0.993	1.000-1.004 / 1.000	0.991-1.000 / 0.988	0.968-1.023 / 1.040
Intercept									
cobas t 711[b] / cobas t 511[c]	0.132-0.337 / 0.444	-0.0431-1.21 / -0.052	-0.100-0.454 / 0	-0.00642-0.00799 / 0.00145	-12.1-0.0774 / -0.00145	-2.00-2.75 / 0.0987	-0.05000-0.0133 / 0.100	0.000-0.0313 / 0.0702	-0.476-0.525 / -1.18

[a]Results for control samples with the same acceptance criteria are pooled, results for control samples with difference acceptance criteria are presented separately (D-Dimer and thrombin time); [b]**cobas t** 711 analyser tested at three sites; [c]**cobas t** 511 analyser tested at one site.
aPTT, activated partial thromboplastin time; PT, prothrombin time

A-298

Discriminating Red Cells, White Cells and Platelets in Chordate Blood Samples using a Simple Detection System and a Single Analysis Cycle

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Background: We show that Red Cells (RBC), White Cells (WBC), and Platelets (PLT) can be discriminated in a single analysis cycle by an optical system consisting of only a 405 nm laser, a flow cell, and two detectors; one parallel to the flow cell front face collecting a 23 degree cone of light about incidence and another parallel to a side face collecting 70-110 degrees. No lenses are required for the detectors. The system works for all chordates, regardless of RBC size, shape or nucleation. Discrimination is based on absorption of 405 nm light by hemoglobin, present only in RBC, and WBC and PLT side scattering patterns. More than 95% of light scattered by a blood cell remains within a 23 degree cone. Therefore the front detector signal is dominated by absorption. Cells do scatter <5% of light outside 23 degrees, generating "pseudo-absorption" signals, so that WBC pseudo-absorption signals overlap RBC absorption signals if MCV<30 fL. Therefore a side scatter detector is also used, to distinguish between WBC and small RBC. For samples with MCV>30 fL, only the absorption detector is needed. PLT produce such small pseudo-absorption signals that they do not overlap even very small RBC signals. **Methods:** This method is a significant improvement over current flow cytometric methods that rely on light scattering, in terms of both simplicity and application range. The total scattering intensity relationships: WBC>RBC>PLT should provide discrimination. However, the cell concentration ratio in e.g. humans is typically 450 RBC/25 PLT/1 WBC. To count sufficient WBC in a diluted unlysed whole blood sample quickly enough to be commercially useful, RBC counting frequency would produce 3%-7% signal doublets. These would overlap WBC signals, and WBCs could not be counted. A separate analysis cycle involving RBC lysis is therefore required. Even this approach does not work for bird, fish, and reptile WBC because the lysed RBC release nuclei, generating multiplets that overlap WBC.Our method is not subject to RBC/WBC signal overlap; in fact, RBC doublets are better discriminated from WBCs than single cells, since more HGB is detected. Blood samples can be analyzed in a single cycle even at very high concentration;

e.g. 50:1 dilution, with cycle time <60 seconds. The method applies regardless of RBC shape and to nucleated-RBC species as well as to mammals, because absorption signals are only weakly sensitive to shape and all RBCs contain hemoglobin. **Results, Conclusion:** We have analyzed samples whose MCV range from 16 fL to 325 fL, including human, other mammalian, bird, and reptile samples, and shown discrimination for all samples. RBC- and WBC absorption channel signals are detected, while PLT signals are too small to be detected in this version of the system, even for bird and reptile samples which have large thrombocytes. PLT can be detected in an expanded dynamic range version.

A-299

Development of a liquid fibrinogen calibration verification set to verify the method's linearity and validate the analytical measurement range (AMR).

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Introduction: Fibrinogen, a 340kDa plasma glycoprotein, is a primary component of blood clots. Reduced levels may contribute to bleeding risk. Increased fibrinogen consumption in cases of disseminated intravascular coagulation (DIC) or decreased fibrinogen synthesis by liver disease, may result in decreased fibrinogen levels. Fibrinogen assays have been cleared by the U.S. FDA as non-waived laboratory tests, therefore, calibration verification is required under CLIA '88. Our objective was to develop a liquid-stable, human plasma fibrinogen test kit to meet calibration verification needs of clinical laboratories. The kits are for the Instrumentation Laboratory (IL) ACL TOP® 500, Siemens CS 2500 and the Stago Compact Max. **Methods:** VALIDATE® Fibrinogen, prepared in a human plasma matrix, was formulated according to CLSI EP06-A into five equal-delta concentrations to cover the manufacturer's analytical measuring range (AMR) for fibrinogen. Manufacturing and recovery targets were adjusted to meet the manufacturer's reportable range: IL (30 to 1000 mg/dL), Siemens (80 - 450 mg/dL, CCR 50 - 860 mg/dL) and Stago (150 - 900 mg/dL). The CLSI EP05-A3 guideline for Evaluation of Precision of Quantitative Measurement Procedures was followed. The three different calibration verification formulations were tested on their respective systems at two separate sites for reproducibility and precision assessments. Linearity was assessed, using samples tested in triplicate, per level. Linearity was evaluated using MSDRx® (LGC Maine Standards' proprietary linearity software). **Results:** For the IL ACL TOP® 500, reproducibility results for Levels 1 through 5 (n = 75/level) ranged from 6 to 8 total %CV. Precision results for Levels 1 through 5 (n = 240/level) ranged from 6 to 10 total %CV. For the Siemens CS 2500, reproducibility results for Levels 1 through 5 (n=75/level) ranged from 3 to 10 total %CV. Precision results for Levels 1 through 5 (n=240/level) ranged from 2 to 6 total %CV. For the Stago Compact Max, reproducibility results for Levels 1 through 5 (n = 75/level) ranged from 3 to 4 total %CV. Precision results for Levels 1 through 5 (n =240/level) ranged from 3 to 7 total %CV. All formulations demonstrate a linear response on their respective platforms and have excellent reportable range coverage. **Conclusion:** VALIDATE® Fibrinogen, as a liquid, five-level, ready-to-use test kit, is effective for calibration verification testing and reportable range method validation. Each formulation provides coverage of the respective manufacturer's claimed reportable range for the fibrinogen assay. The IL, Siemens and Stago formulations are currently available and stability studies are on-going. All formulations are listed with the FDA.

A-300

Multicenter Study of the High-volume Sysmex CS-5100 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers*

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Background: The objective of this study was to compare the performance of two automated coagulation analyzers, the high-volume Sysmex® CS-5100 System (CS-5100) and the Sysmex CA-1500 System (CA-1500), using reagents from Siemens Healthineers. Instrument performance for factor V Leiden (FVL), factor II deficiency (FII), factor VIII deficiency (FVIII), factor IX deficiency (FIX), factor X deficiency (FX), factor XI deficiency (FXI), factor XII deficiency (FXII), and lupus anticoagulant (LA screening [LA1], LA confirmation [LA2], and LA ratio [LAR]) were compared. **Methods:** A measurement comparison (MC) study was performed according to CLSI EP09-A3E (*Measurement Procedure Comparison and Bias Estimation Using Patient Samples*). Four clinical sites or internal measurements were included for testing de-identified leftover samples. The MC of the CS-5100 versus the CA-1500 was based on 165–495 results per parameter (total of 3136 results). A reproducibility study was performed according to the CSLI EP05-A3 (*Evaluation of Precision of Quantitative Measurement Procedures*) guideline in three laboratories. Between five and seven samples were measured covering medical decision points and the clinical reportable ranges for each test. The complete dataset contained 14,968 results. Additional performance data were determined for regulatory clearance. **Results:** Results correlated well between the CS-5100 and the CA-1500. The MC studies showed Passing-Bablok regression slopes ranging from 0.94 to 1.05 and Pearson correlation coefficients ≥0.966 (depending on the application). Reproducibility testing for the new device/test combinations showed low CV values. The mean reproducibility (total CV combined labs) of all samples and parameters was 3.7%, ranging from 0.9 to 7.3% (depending on application and sample). **Conclusion:** The CS-5100 compares well to the CA-1500 and offers the benefits of state-of-the-art functionality and ease of use in high-volume coagulation laboratories. *Product availability varies by country. Sysmex is a trademark of Sysmex Corporation.

A-301

Acute promyelocytic leukemia with atypical presentation and fatal outcome. Case description and literature review.

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Background: Acute promyelocytic leukemia (APL) represents about 25% of all acute myeloid leukemia in Latin America. There are two morphological variants, hypergranular (M3) and microgranular (M3v); the latter is rare and it is often confused with myelomonocytic leukemia (M4). Phenotypically more than 94% of the cases are positive for the CD117 antigen and above 95% and 75% are negative for HLA-DR and CD34 respectively. Just over 95% of the cases present translocation (15;17), which confers susceptibility to conventional pharmacological treatment, while about 3% of patients express the chimeric protein ZBTB16 / RARA, resulting from the t(11;17) and are resistant to conventional treatment. **Objective:** Description of a M3v-APL, CD117 negative / HLA-DR positive, t (11; 17) positive with fatal outcome. **Case description:** A 40 year old male with a persistent headache, ecchymosis in the right arm and infiltrative lesion on the palate, whose blood test results showed anemia, thrombocytopenia and leukocytosis (89,000 leukocytes per µL) along with 71% blasts of myelomonocytic appearance in the peripheral blood. The bone marrow cells showed similar characteristics to those found in peripheral blood. In order to classify the leukemia and establish the prognosis flow cytometry and molecular analysis were performed. The immunophenotype showed 96% of myeloid blasts and a treatment with all-trans retinoic acid (ATRA) was initiated. In a computerized tomography scan a hyperdense image in the right frontal region suggestive of leukemic infiltration was observed. It was not possible to perform a lumbar puncture. The patient suffered right parenchymal hematoma and uncal herniation, dying on the next day.

Method: Immunological phenotype based on the identification and quantification of cellular antigens by means of flow cytometry. Genetic abnormalities assessed by reverse transcription polymerase chain reaction (RT-PCR) for simultaneous detection of six myeloid leukemia translocations. **Results:** Blasts cells were negative for CD117 and CD34, with heterogeneous expression of CD36, CD64 and HLA-DR. RT-PCR revealed thet (11;17) confirming the diagnosis of APL. **Discussion:** Different reports describe atypical APL as M3v leukemia with an unusual antigenic expression pattern or a rare translocation. In a series of 20 cases with APL M3v all were CD117 positive and HLA-DR negative. In another series with 16 cases of M3v APL, 12 were positive for CD117 and only 1 for HLA-DR. The expression of the CD36 antigen has not been described to our knowledge in any APL report. The combination of CD36 and CD64 is used for the identification of leukemia with monocytic differentiation. Despite its low frequency, t (11; 17) has only been described in APL with habitual phenotypic expression. **Conclusion**: This case confirms that t (11; 17) can occur in an APL with morphological and phenotypic characteristics similar to myelomonocytic leukemia. Fatality was associated with the genetic alteration responsible for clonality and resistance to conventional treatment with ATRA.

A-302

Reliability of automated platelet counts in moderate to severe thrombocytopenia. comparison of optical with impedance methods

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BACKGROUND: Patients with thrombocytopenia (platelet<150x10^3/uL) require accurate and reliable platelet counts for effective clinical assessment and management. Current methodologies for automated platelet counting include impedance and optical light scatter. Limitations exist with the impedance method as microcytes and fragments maybe ascribed as platelets. **OBJECTIVE:** We aim to investigate the impact of different platelet counting methods in moderate and severe thrombocytopenia. **METHODS:** Full blood counts were performed on the Sysmex XN-9000 hematology analyzer, with our primary platelet count method being optical light scatter. We compared the platelet counts of 105 patients (64 male, 41 female) with moderate (50-100x10^3/uL) and severe (0-50x10^3/uL) thrombocytopenia by replicate analyses using the impedance and optical counting modes on the XN-9000. Statistical analyses were performed using MedCalc v16.0 (MedCalc Software, Ostende, Belgium). **RESULTS:** In subjects with normal platelet counts, there was close correlation between platelet methods with impedance giving higher values than optical (R=0.964). This discrepant platelet counts were more pronounced in moderate (R=0.831) and severe (R=0.905) thrombocytopenia. Optical platelet counts agree closely with the optical fluorescence platelet method (R=0.983). **CONCLUSION:** The optical platelet modality on the XN9000 showed good agreement with the fluorescent method. Platelet counts on the impedance mode shows a positive bias of 6.3 to 11.5% with respect to the optical mode. The platelet methodology employed in practice may impact optimal clinical management especially in states of thrombocytopenia.

A-303

Early urinary biomarkers of renal damage in sickle cell disease patients

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Background: Sickle cell disease is a genetic monogenic disorder with variable clinical manifestations and is characterized by the presence of hemoglobin S. Renal damage is common in patients with this disease which begins in childhood, progressing with age, a fact that makes nephropathy one of the possible complications. This can compromise patients' quality of life, and result in decreasing survival. Classical biomarkerssuch as creatinine, are not able to detect early renal lesions which makes kidney injury molecule-1 (Kim-1) and N-acetyl-β-D-glucosaminidase (NAG) possible biomarker candidates in the prediction of renal disease in sickle cell anemia patients. The aim of this study was determine KIM-1 and NAG urine levels in sickle cell anemia patients and in a control group. **Methods:** It was a cross-sectional study with a total of 56 individuals up to 17 years of age, 32 of whom had sickle cell disease and 24 healthy individuals. All were treated at the Laboratorio de Analises Clinicas da Faculdade de Farmácia da UFBA. The study was approved by the local ethical committee and blood samples were collected after signing informed consent forms. Hematological analyses were performed with automated protocols. KIM-1 and NAG urine levels were investigated by immunoenzymatic assay (ELISA) as per manufacturer protocols (USCN, USA). All data were analyzed in SPSS version 24, considering p <0.05. **Results:** The results showed that KIM-1 and NAG levels were significantly higher (p<0.0001 for both) in the patients group, compared with the control group (6.95 ± 7.13pg / mL vs 1.72 ± 1.87 pg / mL for KIM-1 and 0.43 ± 042ng / mL vs 0, 07 ± 0.11 ng / mL for NAG). **Conclusion:** KIM-1 and NAG levels were higher in the patients group, which suggests it may be incorporated in a follow up of sickle cell anemia patients in focus identify early renal damage.

A-304

Installation of Two TEG® Instruments in a Clinical Laboratory. Detection and Corrective Actions when Alpha Angle Level I Control Showed an Unnatural Behavior.

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Background. The thromboelastogram offers to the trauma surgeon both diagnostic information on blood clot formation deficiencies and it monitors the effect of therapeutic interactions. We report the detection of unnatural behavior of one quality control (QC) parameter, namely alpha angle, while installing two TEG® 5000 analyzers and the subsequent corrective actions. **Materials.** Two TEG® 5000 instruments (Haemonetics®), QC material (Level I, lot #1101-1201, HMO #3137; Level II, lot #1022-1202, HMO #3132, Haemonetics). The short-term precision was evaluated by assaying both levels of QC material with each instrument five times throughout the day for five consecutive days. This was performed by the manufacturer's representative (SS) and one of us (KB). The long-term precision was evaluated by assaying both levels of control material with both instruments for thirty days. The assays were performed by the technologists assigned to each of the three daily shifts. The data were transferred manually from the instruments printout to Minitab® (version 17 Minitab Inc.) statistical software. **Results.** We present the results for alpha angle control material. The short term precision showed for both levels of controls quasi-normal distribution (normal probability plots), independence (P>0.05), equality of standard deviations (Multiple comparisons P>0.05, Levene's P>0.05), and of daily means (ANOVA by day P>0.05, Instrument P>0.05). The long-term precision showed that while for angle level II the data showed a natural pattern as observed for the short-term precision study, for angle level I the data showed a negatively skewed distribution (skewness = -3.44), the histogram and the normal probability plot showed a shift to the left. The parallel box plot by technologist showed differences for mean performance and variability (length of the whiskers). ANOVA showed statistically significant differences (P<0.05) by technologist and multiple comparisons (P<0.05) and Levene's (P<0.05) tests with the Bonferroni's 95 % C.I. showed statistically significant differences between technologist's standard deviations. Upon obtaining new, calibrated pipettes (Pipet-Lite XLS®, Raining), an anti-vibrational table, and intense technologists training (performed by two members of the team (SS and KB) the unnatural distribution of the QC data was corrected as demonstrated by the quasi-normal distribution, independence, equality of the technologists means (ANOVA, P>0.05) and standard deviations by technologists (multiple comparisons (P<0.05) and Levene's (P<0.05) tests) for the data collected during 30 days of operation. **Conclusions.** The TEG technology requires good manual dexterity and a vibration free surface. The assistance of the manufacturer's representative (SS) was critical in training the younger technologists who are more adapted to hands-free methodologies. The availability of QC statistical software such as Minitab, was critical for analysis and interpretation of the data.

A-305

Acute Myeloid Leukemia with Erythrophagocytosis in Peripheral blood smear and marrow bone: case report

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Background: Patient 28 year-old woman was admitted to our Institute emergency service with the diagnosis of Acute Myeloid Leukemia (AML) with monocytic component, the patient presented loss of alertness, radiological suspicion of infiltration to the central nervous system and magnetic resonance with subarachnoid hemorrhage, at diagnosis with 43% of blasts in hemodiluted bone marrow aspirate. During admission she developed febrile peaks, a thorax tomography was performed in which a bilateral basal infiltrate was evident, suggestive of a multiple foci infectious process, a treatment with piperacillin-tazobactam and vancomycin was initiated. Since admission she presented headache, infectious complications with pulmonary Aspergillosis, herpetic

gingivostomatitis, transvaginal bleeding, sphenoidal and frontal sinusitis. At admission she had anemia, neutropenia and thrombocytopenia, she was on chemotherapy treatment and multiple transfusions of blood components. Since she was diagnosed with AML she had three relapses; June 2014, April 2016 and March 2017. She was in protocol for bone marrow transplantation, but due to the poor evolution and lack of a donor, transplant was not performed. Finally the patient died, her diagnostic end was hypoxemia refractory. **Results:** Hemostasis: Prothrombin time 11.4 s (normal) aPTT 22.4 s (low). Hemogram: Leukocytes $4.7x10^3$, Hemoglobin 7.2 g / dL, Platelets $4.0x10^9$ / L, 48% blasts with isolated images of erythrophagocytosis, presence of Auer rods and hypogranular neutrophils. Most recent bone marrow aspirate: 48% blasts (IF> 20% myeloid blasts). Immunophenotype: CD45dim/ CD34 positive / CD13 positive / CD117 positive / HLADR positive / CD33 positive, MPO positive, CD7 positive. Cytogenetic Study: 11 metaphases 45, X, -X, der(3) t(X;3) (q29;p11.2), t(8,21) (q22; q22). **Conclusion:** Erythrophagocytosis is a rare finding in acute leukemias described in less than 1% of cases. In these, erythrophagocytosis has been associated with some subtypes of AML, especially with the M4 and M5 subtypes (according to the FAB classification). These subtypes have been associated with alterations involving the C-MOZ gene, located in chromosome (8) (p11), the most frequent alteration being t (8; 16) (p11;p13); in this translocation the C-MOZ gene in (8)(p11) is rearranged with the CBP gene in 16p13 giving rise to the C-MOZ / CBP7 fusion gene. The second less frequent translocation is t (16; 21). The exact mechanism by which leukemic blasts phagocytose erythrocytes is not well known. It has been postulated that it could be related to the aberrant premature expression of complement receptors CR1 and CR3 and of the FcR receptors of IgG and gp150. Another hypothesis is that all these alterations could be stimulated by the coexistence of disseminated intravascular coagulation. This case is very interesting because it is the first case described in Mexico. Leaves context for being an AML with erytrophagocytosis t(8;21), that is not described in the clinical cases reported in the literature.

A-306

Comparison of Advia 120 CHr with the Abbott Alinity H MCHr as a potential analyte in patients with Chronic Kidney Disease

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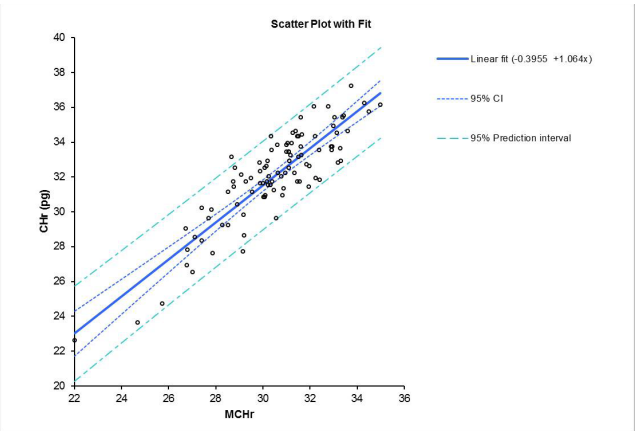
Functional Iron Deficiency (FID) exists in chronic kidney disease where there are adequate iron stores but a failure to incorporate the iron into the developing erythron. Mean cell haemoglobin (MCH) and mean cell volume (MCV) vary over a period of weeks and may not be useful in monitoring FID or its treatment. Reticulocyte haemoglobin (CHr or Ret-He) gives a direct estimate of the availability of functional iron over days, rather than weeks, and is useful for monitoring acute changes in renal patients receiving Erythropoiesis stimulating agents. A CHr value <29 pg predicts for iron response in patients with chronic kidney disease (CKD) receiving ESA therapy. In this study the CHr and MCHr were measured and compared on the ADVIA 120 (CHr), and the Abbott Alinity H (MCHr) respectively. The Alinity H analyser is a new high throughput analyser from Abbott. 99 whole blood EDTA samples with an MCH of ≤ 34pg in were chosen. Samples were aliquoted in two allowing simultaneous testing on both analysers. The results showed excellent linear correlation between the two datasets with a consistent positive bias of the CHr over the MCHr. These results fit the equation for Linear fit and the Altman Bland test gave a p value of <0.0001. Using the linear fit equation of MCHr = (Chr+0.3995)/1.064 a CHr of 29pg is equivalent to 27.6pg. Given this and clinical utility of the CHr, using an MCHr cut-off of 27.6pg may be used to predict iron responsiveness in patients with CKD in laboratories using the Alinity H analyser. References: NICE: Chronic kidney disease: managing anaemia. June 2015 Update. **National Institude for Health and Care Excellence,** NG8, 2015. THOMAS, D. W. et al. Guideline for the laboratory diagnosis of functional iron deficiency. **British Journal of Haematology,** 161, (5), p. 639-648, 2013.



A-307

IgD Multiple Myeloma: clinical features and prognosis

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Background: IgD Multiple Myeloma (MM) is a rare variant (2% of all MM) associated with a poor prognosis. The aim of our study is to describe the clinical and analytical characteristics of patients with MM IgD in our geographical area and to highlight the fundamental role played by the clinical laboratory in the study of these patients. **Methods**: Five patients diagnosed of IgD-MM between 2010 and 2015 have been studied. Data were analyzed from the medical record of the patients. **Results**: The main presenting features of the patients were: median age at disease presentation of 63 years, male predominance (80%), bone pain (80%), lytic bone lesions (100%), renal function impairment (100%), creatinine of 3,37 mg/dl, predominance of lambda light chain (80%), M-protein undetected by serum protein electrophoresis (40%), serum free light chains ratio abnormal at baseline (100%), hypercalcemia (40%), presence of plasmacytomas (20%), associated amyloidosis (20%) and aggressive clinical course (ISS-3, 80%). The median value of plasma cell in bone marrow was 26%. In the patient with IgD Kappa MM; the value of free kappa at diagnosis was 24769 mg/L with free lambda of 15.78 mg/L and a ratio of 1570. In IgD Lambda MM patients; the median value of free lambda was 2859 mg/l with median free kappa of 7.57 mg/L and a median ratio of 0.002. There were four disease-related deaths with a short median survival of 21 months.

Patient	**1**	**2**	**3**	**4**	**5**
Age (years)	83	50	63	54	77
Plasma Cells (%)	28	4	15	60	26
Serum Protein Electroforesis	Positive Large peak	Negative	Negative	Positive Small peak	Positive Small peak
Serum Immunofixation	IgD-L	IgD-K	Negative	IgD-L	IgD-L
Bence Jones Protein	No sample	Kappa	Negative	Lambda	Lambda
Free Kappa (mg/L)	11.20	24769	1.57	3.15	8.62
Free Lambda (mg/L)	1410	15.78	3290	4025	2427.5
Ratio K/L	0.008	1570	0.0005	0.001	0.0036
Beta-2-Microglobulin (mg/L)	23.06	21.82	3.0	6.2	18.7
Creatinine (mg/dL)	8.3	14	1.20	4.24	3.37
Diagnosis	IgD-L MM ISS 3	IgD-L MM ISS 3	IgD-L NSMM ISS 1	IgD-L MM ISS 3	IgD-L MM ISS 3
Survival (months)	1½ (Exitus)	21 (Exitus)	48 (Exitus)	10 (Exitus)	5 (Alive)

Conclusions: IgD MM presents clinical and laboratory findings that defines a distinct entity. In our population, IgD MM is characterized to have poor prognosis with a median survival of 21 months after diagnosis confirming previous studies. The recognition of IgD monoclonal component can be sometimes difficult to detect by SPE and the quantification of serum free light chains are essential for the diagnosis of these patients and improve the management of patients these patients.

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Distinct Biomarkers Reflect Pathophysiological Differences of Sickle Cell Disease (SCD) Sub-phenotypes: Viscosity-Vaso-Occlusion vs. Hemolysis-Endothelial Dysfunction

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Background: Patients with SCD have variable phenotypes, with different severity of pain and other symptoms, including lung injury, stroke, leg ulcers, renal injury, osteonecrosis, and systemic pulmonary hypertension. Recently, two distinct sub-phenotypes have been defined: a) Patients with the Viscosity-Vaso-Occlusion sub-phenotype (VVO) suffer mainly from vaso-occlusive pain crises with a relatively high hemoglobin concentration and b) patients classified as the Hemolysis-Endothelial-Dysfunction sub-phenotype (HED) suffer from stroke and pulmonary hypertension with an elevated concentration of LDH. We aimed to explore the correlation of key biomarkers with the two sub-phenotypes of the disease, namely: Placental Growth Factor (PlGF) a member of the Vascular-Endothelial-Growth-Factor superfamily, which plays an important role in both inflammation and neo-angiogenesis; vWF:antigen, a multimeric plasma glycoprotein secreted by the endothelium; Growth-Differentiation-Factor-15 (GDF-15), a member of the TGF-β superfamily, which expression is strongly upregulated in response to oxidative stress, inflammation, tissue injury and in conditions related to ineffective erythropoiesis. We tested these biomarkers in patients with compound heterozygous SCD and beta-thalassemia (HbS/βthal). **-Methods**: Ninety adult Caucasian patients with HbS/βthal were included in the study, while 20-apparently healthy individuals, served as controls. Patients with HbS/βthal were divided in two groups according to their LDH levels: High-LDH (LDH>270U/L) (HED-phenotype) group (42-patients) and the Normal-LDH (LDH<270U/L) (VVO-phenotype) group (48-patients). Along with hematologic and blood chemistry parameters determination, measurements of circulating levels of PlGF, vWF:antigen, GDF-15, hs-CRP, Cystatin C, hs-TnT and D-Dimers were performed in both groups of patients and controls. **Results**: We found that patients with the HED-phenotype compared to the VVO-phenotype of the disease had lower Hb levels ($p<0.001$), higher Reticulocyte-Production-Index and higher bilirubin ($p<0.001$ and $p=0.004$, respectively), while there were no differences regarding HbF levels between the two groups. PlGF levels were significantly elevated only in patients with the HED-phenotype (22.6±7.1pg/mL) compared to the controls (15.2±2.4pg/mL) ($p<0.001$) and patients with the VVO-phenotype (18.2±7.6pg/mL) ($p=0.005$). vWF:antigen concentrations were markedly elevated in both groups of patients compared to controls (186.4±81.7 and 157.8±73.4 vs 85.3±22.1IU/dL, $p<0.001$), with the increase of vWF:antigen levels to be more pronounced in patients with the HED-phenotype ($p=0.008$). Similarly, GDF-15 levels were also markedly elevated in both groups of patients compared to controls (2,346.2±1295.6 and 1,693.5±1398.3 vs 665.4±221.9pg/mL; $p<0.001$), with the increase of GDF-15 levels to be more pronounced in patients with the HED-phenotype ($p=0.006$). We found also significant higher levels of D-Dimers in patients with the HED-phenotype ($p<0.001$) compared to patients with the VVO-phenotype, while no differences were found in parameters of inflammation and renal function. **Conclusions**: These findings demonstrate for the first time the correlation and involvement of PlGF, vWF:antigen and GDF-15 in the pathophysiological mechanisms of the HED sub-phenotype in patients with the HbS/βthal. Although, there is a degree of overlapping between the two sub-phenotypes of SCD, the differences in the specific biomarkers were significant. Thus, these markers along with the clinical profile could better identify the two subtypes of SCD patients and drive an innovative approach with the use of direct personalized therapies for each specific sub-phenotype by targeting the predominant mechanism in this multifactorial disorder.

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Simultaneous Validation and Verification of Multiple Instrumentation Laboratory ACL TOP Coagulation Analyzers in a Clinical Core Laboratory

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Background: Due to a Mayo Clinic Clinical Laboratory coagulation testing standardization process, multiple Instrumentation Laboratory (IL) ACL TOP Family 50 Series LED optical systems were chosen to replace greater than 10 years-old electromagnetic mechanical end-point clot detection system analyzers (Stago) in the core laboratory. The performance of ACL TOP 750 and 550 analyzers was compared to existing Stago analyzers during validation of prothrombin time (PT), International Normalized Ratio (INR), activated partial thromboplastin time (APTT), thrombin time (TT), D-Dimer, fibrinogen (Fib) and heparin anti-Xa (anti-Xa) testing. Here, we describe the processes used to verify the IL ACL TOP manufacturer performance characteristics. **Methods:** A comprehensive validation was performed to verify manufacturer performance characteristics of the ACL TOP Family 50 Series instruments for PT, INR, activated APTT, TT, D-Dimer, Fib, and anti-Xa testing. The focus of the validation was to verify the accuracy (ACL TOP versus Stago method comparison and ACL TOP versus ACL TOP instrument comparison), precision, and reportable range of these tests. A minimum of 30 samples were used for each test method comparison. Slope (0.90-1.10), correlation coefficient (>0.90), and percent and absolute differences (manufacturer stated) were used as criteria for acceptability. Precision testing was performed by testing each control five times within a run for five days, and percent coefficient of variation was calculated (acceptable ranges were <5% CV for PT and TT, and <10% CV for APTT, D-Dimer, Fib, anti-Xa). The IL stated reportable range for each test was verified. Linearity testing was performed for Fib, D-Dimer, and anti-Xa assays. Slope (0.90-1.10), R^2 (>0.95) and percent recovery (90-110%) were used as acceptance criteria for linearity testing. Results were organized by instrument and test and entered into spreadsheets in real time to allow for faster data evaluation and troubleshooting. **Results:** Other than TT and APTT, method comparison results for all R^2 values were within limits. Anti-Xa, APTT, and INR slope results were slightly outside acceptable limits, but the remainder of the results were within acceptable limits. With the exception of TT, the average percent difference results were within acceptable limits. TT testing showed larger differences (IL ACL TOP versus Stago TT tests) due to the different sensitivities of the reagents in each system to heparin and fibrinogen abnormalities. Observed differences in slope and R^2 for APTT testing were also likely due to the different methodologies in the two systems. ACL TOP to ACL TOP instrument comparisons showed good correlation, with all results within acceptance limits. Precision results were all acceptable. Linearity results for fibrinogen and anti-Xa were within acceptable regression and recovery limits. D-dimer results were slightly outside of recovery limits for two samples, but these differences were not clinically significant. **Conclusions:** Although verification of new analyzers is a common process, unique challenges were encountered during the validation process of multiple ACL TOP Family 50 Series analyzers undertaken to replace current outdated coagulation analyzers. However, our clinical laboratory was able to overcome these obstacles through identification of organizational steps that helped to streamline the validation processes and the transition to the new coagulation analyzers.

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Screening for Light Chain Glycosylation: A Potential Route to Earlier Diagnosis of AL Amyloidosis

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Background:
Immunoglobulin light chain (AL) amyloidosis is a rare plasma cell disorder affecting ~10 people/million people/year. AL amyloidosis is characterized by abnormal secretion of immunoglobulin light chains (LCs) from clonal bone marrow plasma cells. Secreted clonal LC misfolds into aggregates, forming fibrils in organs like kidney, heart, liver, etc. Serum free light chain (FLC) ratios are the most sensitive method to detect LCs in AL patients, but given that 45 percent of patients with monoclonal gammopathy of unknown significance (MGUS) also have abnormal FLC ratios, abnormal FLC ratios have low specificity for AL. Hence, there is a critical need for a serum-based diagnostic assay that can stratify AL risk with high specificity, which enables accurate and earlier diagnosis of AL with potential to improve patient outcomes.
Methods:

Serum samples were collected from 189 AL patients (128 AL-lambda, 61 AL-kappa) and 122 non-AL patients (distributed among 54 multiple myeloma, 57 MGUS, 8 Waldenstrom's macroglobulinemia and 3 other plasma cell disorders, with a 2:1 ratio of kappa:lambda M-proteins). Serum LC was purified using nanobody-enrichment and analyzed by MALDI-TOF-MS[1]. LC mass spectra were visually analyzed for abnormally broad complex patterns that are generated by N-glycosylation. Kappa and lambda LCs with potential N-glycosylation were deglycoslylated with PNGase F and N-glycosylation was confirmed by looking for a shift in LC mass (due to deglycosylation) via MALDI-TOF-MS and LC-ESI-Orbitrap MS.
Results:
33% of AL-kappa patients had a suspected LC glycosylation pattern when compared to only 10.2% of AL-lambda patients. The rate of LC glycosylation among non-AL patients was only 4.1%; rate among kappa non-AL patients was 3.7% and among the lambda non-AL patients was 4.9%. In order to confirm that the broad peak pattern observed for native LCs is due to N-glycosylation, 21 kappa M-protein (18 AL and 3 non-AL) and 9 lambda M-Protein (7 AL and 2 non-AL) LCs were deglycosylated with PNGase F and LC mass spectrum were re-examined by MALDI-TOF-MS and LC-ESI-Orbitrap. In all samples, the abnormally broad complex peak patterns observed in native LC spectra were shifted into a monoclonal LC peak, confirming the presence of N-glycosylation in native LCs. The odds of glycosylated kappa LC patient being AL was 12.68 ($p<0.0001$), glycosylated lambda LC patient being AL was 2.20 ($p=0.31$), and LC glycosylation being AL was 4.95 ($p=0.0012$).
Conclusion:
This work confirms the over representation of LC glycosylation in AL patients when compared to other plasma cell disorders. The frequency of kappa LC glycosylation was 5 times higher than lambda in AL patients. The odds of a glycosylated LC being AL was very high and clinically actionable. In addition, the MALDI-TOF-MS method used to detect LC glycosylation is currently being validated for clinical use; allowing for rapid assessment of AL risk in MGUS patients. This potentially could lead to earlier clinical suspicion of AL in MGUS patients with glycosylated LCs.
Reference:
1. Milani P, Murray DL, Barnidge DR et. al. The utility of MASS-FIX to detect and monitor monoclonal proteins in the clinic. Am J Hematol. 2017: 92: 772-779.

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Precision and method comparison data for three new coagulation assays measuring activated partial thromboplastin time (aPTT) on the new cobas t 711 and t 511 analysers

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Background: Assessment of aPTT is a good indicator of intrinsic coagulation pathway activity and is widely used to diagnose coagulopathies and monitor anticoagulant therapy. The objective of this multicentre study was to evaluate the precision of three coagulation assays (aPTT, aPTT Lupus and aPTT Screen) with different sensitivities to heparin, lupus anticoagulant and factor deficiencies, and compare their performance against commercially-available assays/platforms.
Methods: Anonymised human 3.2% sodium citrate plasma samples were used (commercially sourced or residual clinical). For each assay, within-run precision was evaluated in a single run comprising three controls and five pool plasma samples covering the relevant measuring ranges (21 replicates per sample); reproducibility was evaluated over five days. Method comparisons were performed on the **cobas t** 711 versus reference reagents on the Siemens Sysmex CS or Stago STA-R Evolution systems (≥120 samples per assay, per site); Pearson's r correlation coefficients were estimated. Results were compared against prespecified acceptance criteria.
Results: Across all three assays, coefficients of variance (CV) for within-run precision and total reproducibility were within the acceptance range **(Table)**. CVs for within-run precision were 0.2-1.5% (**cobas t** 711) and 0.2-1.8% (**cobas t** 511); CVs for total reproducibility were 0.4-3.4% (**cobas t** 711) and 0.3-6.3% (**cobas t** 511). Method comparison experiments for all assays (**cobas t** 711) demonstrated excellent correlation versus their respective reference methods, with Pearson's correlation coefficients within the acceptance range **(Table)**.
Conclusion: Each aPTT assay showed good within-run precision, reproducibility, and excellent correlation with commercially-available assays/platforms, thereby demonstrating their suitability for use in core laboratories.

Within-run precision, reproducibility, and method comparison of the aPTT, aPTT Lupus and aPTT Screen

Assay	Within-run precision* (range of % CV)		Total reproducibility† (range of % CV)		Method comparison (cobas t 711)				
	cobas t 711‡	cobas t 511§	cobas t 711‡	cobas t 511§	Reagent	Instrument	n	Acceptance criteria	Pearson's r
aPTT	0.2-1.5	0.2-0.8	0.4-2.9	0.4-3.8	Actin FS	Siemens Sysmex CS	594	≥0.85	0.980-0.986‡
					STA Cephascreen	Stago STA-R Evolution	175	NA	0.819¶
aPTT Lupus aPTT Screen	0.3-1.2	0.2-1.4	0.4-2.9	0.3-2.2	Actin FSL	Siemens Sysmex CS	620	≥0.85	0.967-0.987‡
					STA Cephascreen	Stago STA-R Evolution	99	NA	0.943¶
					STA-LA	Stago STA-R Evolution	128	NA	0.9575¶
aPTT Screen	0.3-1.2	0.2-1.0	0.8-3.1	0.8-6.3	Pathromtin SL	Siemens Sysmex CS	579	≥0.85	0.964-0.985‡
					aPTT	Stago STA-R Evolution	153	NA	0.833¶

*acceptance criteria CV ≤4.0%; †acceptance criteria CV ≤25.0%; ‡range across four sites; §range across two sites; ¶performed at one site only
aPTT, activated partial thromboplastin time; CV, coefficient of variation; NA, not applicable

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Method comparison of the PT Rec coagulation assay with existing reference methodology for measuring prothrombin time (PT)

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Background: Coagulation screening tests are used to reliably and quickly detect inherited or acquired factor deficiencies and monitor patients receiving anticoagulant therapies. New commercially available assays may offer improvements over current assays; however, it is important that new assays are evaluated against existing assays prior to their adoption in the healthcare setting. The PT Rec assay contains thromboplastin (recombinant human thromboplastin reagent containing a heparin-neutralizing substance) and calcium, which initiates activation of the extrinsic coagulation cascade when added to citrated human plasma. The objective of this study was to compare the new PT Rec assay (measure of prothrombin time) with existing reference methodology.
Methods:
Method comparisons, according to Clinical and Laboratory Standards Institute EP09-A3 (CLSI EP09-A3) guidelines, were performed across four sites for the PT Rec assay (**cobas t** 711 analyser) versus Innovin (Siemens Sysmex CS-5100 or CS-2000i). A minimum of 120 residual anonymised human plasma samples were used per assay, per site to represent the appropriate measuring range. Equivalency of the **cobas t** 711 and **cobas t** 511 platforms was also evaluated by method comparison.
Results:
According to prespecified criteria based on Deming regression analyses, method comparison experiments showed good agreement between PT Rec and the reference method (**Table 1**). Equivalency was also demonstrated between the **cobas t** 711 and **cobas t** 511 analysers, according to prespecified acceptance criteria, based on Passing-Bablok regression analyses (**Table 1**).
Conclusion:
Based on good agreement between PT Rec and the commercially available reference, and equivalency observed between the **cobas t** 711 and **cobas t** 511 analysers, the PT Rec assay has demonstrated suitability for use in core laboratories.

Table 1. Summary of method comparison data.

Comparison	Evaluation	Acceptance criteria	Freiburg	Sheffield	Debrecen
			Lot 1	Lot 2	Lot 3
PT Rec versus Innovin[a]	n	-	131	135	130
	Slope (Deming)	1.00 ± 0.10	1.008	1.036	0.9
	Intercept	NA	0.090	0.045	0.238
	Pearson's r	≥0.900	0.9985	0.9966	0.9883
	Bias at 1.0 INR	≤0.15	0.097	0.081	0.138
PT Rec[b]	n	-	129	135	-
	Slope (Passing-Bablok)	1.00 ± 0.10	1.006	0.984	-
	Intercept	NA	0.006	0.033	-
	Pearson's r	≥0.900	0.9999	0.9996	-

[a]Analyses based on INR, method comparison between **cobas t** 711 and reference method; [b]Analyses based on INR, method comparison between **cobas t** 711 and **cobas t** 511 analysers; INR, International Normalised Ratio; NA, not applicable; PT, prothrombin time

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Sensitivity of the aPTT, aPTT Lupus and aPTT Screen assays towards heparin and lupus anticoagulant

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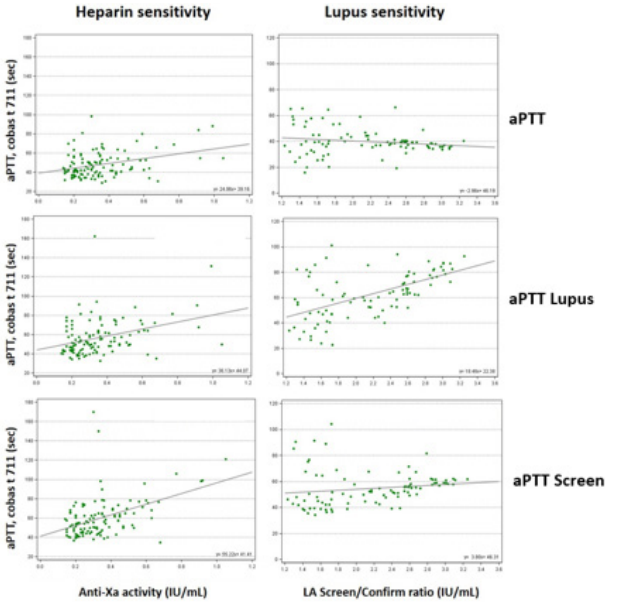
Background: Activated partial thromboplastin time (aPTT) tests are used to monitor anticoagulation therapy and for the assessment of coagulopathies. To assist accurate diagnosis, a range of aPTT assays that give different responses to anticoagulants (heparin) and have varied lupus anticoagulant sensitivity, is required. The objective of this study was to evaluate the sensitivity of three new aPTT assays (aPTT, aPTT Lupus and aPTT Screen) towards unfractionated heparin (UFH) and lupus anticoagulant. The aPTT assay has reduced lupus antibody sensitivity, whilst the aPTT Lupus assay has increased lupus antibody sensitivity. **Methods:** For heparin sensitivity experiments, platelet-poor plasma samples from patients receiving UFH (n=117) were analysed. For lupus sensitivity experiments, lupus anticoagulant-positive commercially available plasma samples (n=96) were analysed. Therapeutic/reference ranges were determined (**cobas t** 711) or data from the package insert were used (Siemens BCS XP); prolongation of clotting times measured using aPTT, aPTT Lupus and aPTT Screen (**cobas t** 711) were then compared with the reference assay (reagents used: Actin FS, Actin FSL, Pathromtin SL). **Results:** Sensitivity to UFH (aPTT Screen > aPTT Lupus > aPTT) and lupus anticoagulant (aPTT Lupus > aPTT Screen > aPTT) varied between assays (**Figure**). Using aPTT Lupus or aPTT Screen, ≥80% of samples from UFH-treated patients within the therapeutic range (anti-Xa activity, 0.3-0.7 IU/mL) resulted in a measurable clotting time. For each assay, prolongation of clotting times in the presence of UFH and lupus anticoagulant were within 0.75-1.25 times that of the reference assay; clotting times for aPTT, aPTT Lupus and aPTT Screen were 0.97, 0.95 and 0.94 with UFH, respectively, and 1.00, 1.04 and 0.96 with lupus anticoagulant, respectively. **Conclusion:** aPTT, aPTT Lupus and aPTT Screen met the pre-specified acceptance criteria for sensitivity to UFH and lupus anticoagulant.



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Derivation of a new reference interval for Reticulocytes counted on an automated platform using the Sysmex XN-9000

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BACKGROUND: The reticulocyte count in peripheral blood reflects bone marrow erythropoiesis and is used in the differential diagnosis of anemia and monitoring bone marrow response to therapy. The current reticulocyte reference intervals for a normal adult population employed in most books and laboratories are 0.2-2.0%. This reference range is historically based on a manual reticulocyte count derived from small samples (usually <100) of "normal" subjects.Manual quantitation of reticulocytes involve staining the ribonucleic acid (RNA) content with supravital stains and counting the number of cells that appear polychromatic or have basophilic precipitate of granules or filaments. **OBJECTIVE:** With a contemporary and more specific automated method of reticulocyte counting, we aim to derive an updated reticulocyte reference interval. **METHODS:** Our current hematology analyzer (Sysmex XN-9000) performs the reticulocyte count using fluorescence flow cytometry. Apart from estimating the reticulocyte count, it also provides information on reticulocyte-hemoglobin and minimizes interference from leucocytes, erythrocyte inclusions and parasites. Reticulocyte counts were performed on the Sysmex from ambulatory subjects with normal hemoglobin (male: 13.0-17.0g/dL; female: 11.5-15.0g/dL) and MCV (76-96fL). Children and pregnant women were excluded.After excluding 31 outliers (Tukey), the distribution of the reticulocyte data was tested for normality (Kolmogorov-Smirnov). Statistical analyses were performed using MedCalc v16.0 (MedCalc Software, Ostende, Belgium). **RESULTS:** The distribution of the reticulocyte counts from our reference population (n=3940, male=2096, female=1844) was non-Gaussian. The derived reticulocyte reference interval (2.5-97.5 percentile) for adults was 0.7% to 2.7%. Gender comparison, tested by non-parametric means (Mann-Whitney U test), showed no significant difference. **CONCLUSION:** Our findings suggest that our reticulocyte reference interval upwards needs to be revised upwards.

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Automated Determination of Leukocyte Cell Population Data in Detecting Neonatal Sepsis

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Background: Neonatal sepsis (NS) is one of the leading causes of morbidity and mortality despite advances in neonatology. Diagnosis which is made by clinical and laboratory findings is challenging because the signs and symptoms are highly variable. Blood culture, considered as a gold standard for diagnosis, has the disadvantages of a low positivity rate and the time delay of 48 to 72 h. Acute-phase reactants such as C-reactive protein (CRP) and procalcitonin (PCT) along with hematological parameters strengthen the diagnosis of NS. Unfortunately none of these tests have been particularly useful in identifying many of the cases. The objective of the study was to evaluate the value of volume conductivity scatter (VCS) parameters of leukocytes in detecting NS and to estimate their optimal cutoff levels using receiver operating characteristic (ROC) curves.**Methods:** The study was conducted at Marmara University Pendik E&R Hospital. A total of 66 babies from neonatal intensive care unit were included, of whom 19 had proven sepsis with blood culture positivity and 22 had clinical sepsis (clinical course consistent with sepsis, culture-negative) with 25 control cases. Complete blood count (CBC) and cell population data including VCS parameters were retrospectively analyzed. VCS parameters had been analyzed by UniCel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). CRP levels and PCT had been determined nephelometrically (Dade Behring BN II, Siemens Laboratory Diagnostics, Germany) and by electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics, Germany), respectively. Statistical analysis was performed using the SPSS software, version 17.0. Results were expressed as the mean±standard deviation. All parameters were compared using analysis of variance (ANOVA). P value<0.05 was considered statistically significant. Cut-off values were established based on ROC curves.**Results:** While CRP and PCT values were not significantly different among all groups, mean neutrophil volume, lymphocyte conductivity SD, mean monocyte volume, and mean monocyte volume SD differentiated sepsis and control cases (P= 0.009; P=0.020; P=0.017, P<0.001, respectively), but these parameters were not different between sepsis and clinical sepsis groups. Lymphocyte median-angle light scatter and upper median-angle light scatter of neutrophils and monocytes were significantly decreased in the clinical sepsis group when compared to the controls (P=0.036; P=0.017; P=0.023, respectively), but these parameters were not different between sepsis vs. control and sepsis vs. clinical sepsis groups (P>0.05). However, upper median-angle light scatter of lymphocytes (MN-UMALS-LY) was significantly decreased in the clinical sepsis group compared to both control and sepsis group. ROC curve analysis revealed 73.2% sensitivity and 81.2% specificity at 56.5 arbitrary units for MN-UMALS-LY in the discrimination of clinical sepsis.**Conclusion:** Early diagnosis in NS may not only prevent delay in treatment and improve the outcome of the infants, but also avoid unnecessary and prolonged usage of antibiotics. This study shows that VCS parameters may provide significant value for the detection of NS, especially in clinical sepsis. When validated by further studies, incorporation of VCS parameters during initial CBC count analysis could be used to provide a timely and convenient sepsis diagnostic tool and lead to early identification of NS.

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Method comparison of cobas m 511 Integrated Hematology Analyzer and Sysmex® XN-10 Automated Hematology Analyzer using samples with targeted medical conditions

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Background: The **cobas m** 511 integrated hematology analyzer (**cobas m** 511 system) is a novel slide-based system that performs a complete blood count, white blood cell differential, reticulocyte count, and nucleated red blood cell count using digital analysis. This single-center study investigated whether the **cobas m** 511 system delivered comparable results to the Sysmex® XN-10 Automated Hematology Analyzer using samples from patients with medical conditions. **Methods:** Laboratory hematology results were reviewed to identify subjects with 23 targeted medical conditions (including hematological malignancies and disorders of cell numbers and function). Residual whole blood samples (n=130) were processed on both systems within 8 hours of venipuncture. Consistent with CLSI EP09-A3, a method comparison was used to assess the correlation and bias between the systems for all parameters. Individual patient parameter results that were valid on both instruments were included. **Results:** All 26 reportable parameters evaluated showed good-to-excellent correlation between the automated results of the **cobas m** 511 system and Sysmex Analyzer, with no significant bias (Table 1; *Data for MCHC, RDW, RDW-SD, %NRBC, #NRBC, %NEUT, %LYMPH, %MONO, %EO, %BASO, %RET, and HGB-RET not shown*). **Conclusions:** The **cobas m** 511 system and Sysmex Analyzer produce comparable results for samples with targeted medical conditions. This demonstrates the robustness of the **cobas m** 511 system when abnormal samples are encountered.

Table 1: cobas m 511 vs. Sysmex Analyzer results

Parameter [units]	Sample range	Pearson's *R*	Intercept	Slope
WBC [10^3/µL]	(0.11–95.41)	0.999	-0.02	0.995
RBC [10^6/µL]	(1.79–7.68)	0.996	-0.01	0.992
HGB [g/dL]	(6.28–17.24)	0.995	-0.31	1.064
HCT [%]	(18.60–56.00)	0.982	-0.53	1.034
MCV [fL]	(69.50–107.80)	0.879	5.35	0.975
MCH [pg]	(20.22–36.48)	0.977	2.87	0.946
PLT [10^3/µL]	(9.00–1379.00)	0.994	-2.02	0.943
MPV [fL]	(8.40–13.00)	0.843	0.82	0.915
#NEUT [10^3/µL]	(0.46–36.82)	0.999	0.03	1.008
#LYMPH [10^3/µL]	(0.16–4.89)	0.985	0.01	0.976
#MONO [10^3/µL]	(0.14–7.65)	0.991	-0.03	1.013
#EO [10^3/µL]	(0.00–1.23)	0.978	0.00	1.031
#BASO [10^3/µL]	(0.00–2.51)	0.962	-0.08	1.829
#RET [10^6/µL]	(0.00–0.34)	0.971	-0.01	0.982

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Accuracy evaluation of low platelet counts on the cobas m 511 Integrated Hematology Analyzer

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Background: The **cobas m** 511 integrated hematology analyzer is a novel slide-based system that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using digital analysis of a microscope slide. This single-center study assessed the accuracy of low platelet (PLT) counts on the **cobas m** 511 compared with the Sysmex® XN-10 Automated Hematology Analyzer and BD FACSCanto™ Flow Cytometer. **Methods:** Residual whole blood samples (n=115) in the PLT range 1-505 x10^3/µL (based on flow cytometer results using CD61-FITC, CD41a-PerCP-Cy5.5, and CD42b-APC antibodies) were randomly processed on the three systems. Sysmex samples were set to automatically reflex to PLT-F function, which is marketed as more reliable for thrombocytic or problematic samples than the standard impedance PLT-I function. Three analyses were performed: **cobas m** 511 and Sysmex Analyzer versus flow cytometer (reference), and **cobas m** 511 versus Sysmex Analyzer (reference). Data were compared using three different thresholds and results included if a valid PLT result was obtained on both systems. **Results:** The **cobas m** 511 demonstrated excellent accuracy compared with flow cytometry, with ≥93.6% sensitivity, specificity, and agreement, and good accuracy compared with Sysmex Analyzer (Table 1). Attempts were made to also study flags for the Sysmex Analyzer PLT-I function however excessive flagging (100% for PLT counts ≤10, 80% for PLT 11-20, and 28% for PLT 21-50 x10^3/µL) indicated these values were unreliable and prevented any further comparison. **Conclusions:** These data demonstrate the robustness and accuracy of platelet counts reported by the **cobas m** 511 system. Many samples were flagged using the Sysmex PLT-I function that, in routine use, would have reflexed to the PLT-F function. All samples on the **cobas m** 511 system had valid PLT results.

Table 1: Accuracy analyses				
	PLT threshold (10^3/μL)	**Sensitivity (%)**	**Specificity (%)**	**Agreement (%)**
cobas m 511 vs. flow cytometer (N = 115)	10	95.5	96.8	96.5
	20	93.6	94.1	93.9
	50	98.6	97.6	98.3
Sysmex Analyzer (PLT-F) vs. flow cytometer (N = 113)	10	100.0	96.7	97.3
	20	100.0	92.5	95.6
	50	100.0	97.6	99.1
cobas m 511 vs. Sysmex Analyzer (PLT-F) (N = 113)	10	88.0	97.7	95.6
	20	94.1	100.0	97.3
	50	100.0	100.0	100.0

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The research of pak1 function in different BCR-ABL subtype in leukemiagenesis and treatment through STAT5 pathway

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Background: BCR-ABL fusion gene has different subtypes such as P210, P190 and is the main aetiological agent for Chronic myelogenous leukeamia (CML) and can also be found in Acute Lymphocytic Leukemia (ALL). Although p21-activated kinase (PAK1) gene is proved to paly an important role in leukemia caused by different BCR-ABL susptype by NimbleGen expression profile microarray analysis and verified by RT-PCR, the mechanism remain unclear. Our research aims to clarify the difference in regulation of PAK1-STAT5 biological axis between different BCR-ABL subtype and its influence for proliferation and apoptosis in leukemia cell and to discuss if there is a synergy or antagonism effect between PAK1 expression and TKI treatment in different BCR-ABL subtype in hope of finding new biomarker and therapeutic targets. **Methods:** PAK1 interference and overexpression lentivirus was constructed and transfered into P210(+) positive CML cellline K562 and P190(+) positive ALL cell line SUP-B15. Western-blot was used to analysis phosphorylation of STAT5,MAPK in cell line treated with pak1 inference/overexpression lentivirus and TKI. BrdU was used to detect cell proliferation and MTT was used to detect cell apoptosis. Flow cytometry was used to detect cell appotosis and proliferation for cell lines treated with PAK1 interference /overexpression lentivirus and TKI. **Results:** WB testing indicated that in K562 cell line, after PAK1 interference, phosphorylation level in STAT5,ERK, JUK have decreased and cells treated with both PAK1 interference and TKI have the lowest phosphorylation level. In SUP-B15 cell line, after PAK1 overexpression, phosphorylation level in STAT5,ERK, JUK have decreased and cells treated with both PAK1 overexpression and TKI have the lowest phosphorylation level. BrdU and MTT test showed that, in K562 cell line, Cell proliferation rate decreased in 24, 48, and 72h after PAK1 interference, and cells treated with both PAK1 interference and TKI have the lowest proliferation rate. In SUP-B15 cell line, Cell proliferation rate decreased in 24, 48, and 72h after PAK1 overexpression, and cells treated with both PAK1 overexpression and TKI have the lowest proliferation rate. Flow cytometry test showed that, in K562 cell line, Cell apoptotsis rate increased and Cell cycle proliferation index(PI) decreased after PAK1 interference, and cells treated with both PAK1 interference and TKI have the highest Cell apoptotsis rate and lowest PI. In SUP-B15 cell line, Cell apoptotsis rate increased and Cell PI decreased after PAK1 overexpression, and cells treated with both PAK1 overexpression and TKI have the highest Cell apoptotsis rate and lowest PI. **Conclusion:** PAK1 is an important differential expression gene between different BCR-ABL subtype. In P210(+) CML, down-regulated PAK1 gene expressions may lead to suppression in cell proliferation and promotion in apoptosis through phosphorylation of STAT5, with a reverse effect in P190(+)ALL, which showed PAK1 might be an an important molecular mechanism of pathogenic difference between different BCR-ABL subtype.In P210 (+) CML, down-regulated PAK1 expression may enhance the effect of TKI, while the reverse in P190(+)ALL, which showed PAK1 might be an an important molecular mechanism of prognosis difference between different BCR-ABL subtype.

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Evaluation of Volume, Conductivity and Scatter Properties of Leukocytes (VCS Technology) as Screening for Infection in Patients Undergoing Therapeutic Hypothermia after Cardiac Arrest

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Background: Induced hypothermia is increasingly applied in patients with cardiac arrest as a therapeutic intervention in intensive care unit (ICU) to prevent ischemia reperfusion injury, resulting in a better neurologic outcome and reduced mortality. One of the underlying mechanisms of the beneficial effects of hypothermia is proposed to be reduction of the inflammatory response. However, a pitfall of reducing the inflammatory response is an increased infection risk. Therefore, we sought to investigate value of VCS parameters of leukocytes and compare it to C-reactive protein (CRP) and procalcitonin (PCT), in the early detection of infection in these patients. **Methods:** A total of 27 adult patients admitted at the ICU after surviving cardiac arrest were included. Twelve patients received standard post-resuscitation care according to the current best practice as control group while 15 patients received the post-resuscitation protocol of the target temperature management (TTM) trial. Blood samples were drawn before initiation of hypothermia, at the 24th hour and at the time the cultures were taken. Presence of an infection was confirmed either by a positive culture (blood, urine, deep tracheal aspirate) result or by the presence of pulmonary infiltrate on chest radiography. VCS parameters were analyzed by UniCel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). CRP levels were determined nephelometrically (Dade Behring BN II, Siemens Laboratory Diagnostics, Germany) and PCT were measured by electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics, Germany). Statistical analysis was performed using the SPSS software, version 17.0. Results were expressed as the mean±standard deviation (SD). All parameters were compared among groups using analysis of variance (ANOVA) and subsequent post hoc range tests. P value <0.05 was considered statistically significant. **Results:** While PCT values didn't differ from the baseline, CRP values were significantly increased at the time cultures were taken, compared to the baseline values in both control and hypothermia group (P=0.18; P<0.001,respectively). There were significant differences for mean axial light loss of neutrophil (MNAL2-NE; P=0.016), SD of volume of lymphocyte (SD-V-LYM; P=0.021), SD of axial light loss of monocyte (SDAL2-MO; P=0.036), monocyte mean volume (MN-V-MO; P=0.009) and SD of volume (SD-V-MO; P=0.015) values at the baseline and at the time cultures were taken in hypothermia group. However, there was no statistical difference between control subgroups for all VCS parameters (P>0.05). **Conclusion:** Infectious complications after cardiac arrest may be more frequent after TTM. Diagnosis of infectious events is complicated in patients after cardiac arrest not only by the physiological effects of TTM but also by the consequences of reperfusion injury and development of postresuscitation disease associated with systemic inflammatory response syndrome. Furthermore, the significance of the usual symptoms of infections is reduced, as well as the value of laboratory markers such as PCT and CRP. VCS parameters which can be obtained easily using an automated blood analyzer, may be promising for helping clinicians in the prediction and early management of infection especially in cardiac arrest patients undergoing TTM. Nonetheless, further research is needed to identify a biomarker with high diagnostic accuracy and validity.

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Multicenter Study of the Mid-volume Sysmex CS-2500 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers*

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Background: The objective of this study was to compare the performance of two automated coagulation analyzers, the mid-volume Sysmex® CS-2500 System (CS-2500) and the Sysmex CA-1500 System (CA-1500), using reagents from Siemens Health-

ineers. Instrument performance for factor V Leiden (FVL), factor II deficiency (FII), factor VIII deficiency (FVIII), factor IX deficiency (FIX), factor X deficiency (FX), factor XI deficiency (FXI), factor XII deficiency (FXII), and lupus anticoagulant (LA screening [LA1], LA confirmation [LA2], and LA ratio [LAR]) were compared. **Methods:** A measurement comparison (MC) study was performed according to CLSI EP09-A3E (*Measurement Procedure Comparison and Bias Estimation Using Patient Samples*). Four clinical sites or internal measurements were included for testing de-identified leftover samples. The MC of the CS-2500 versus the CA-1500 was based on 157–494 results per parameter (total of 3189 results). A reproducibility study was performed according to the CSLI EP05-A3 (*Evaluation of Precision of Quantitative Measurement Procedures*) guideline in three laboratories. Between five and seven samples were measured covering medical decision points and the clinical reportable ranges for each test. The complete dataset contained 14,948 results. Additional performance data were determined for regulatory clearance. **Results:** Results correlated well between the CS-2500 and the CA-1500. The MC studies showed Passing-Bablok regression slopes ranging from 0.92 to 1.04 and Pearson correlation coefficients ≥0.958 (depending on the application). Reproducibility testing for the new device/test combinations showed low CV values. The mean reproducibility (total CV combined labs) of all samples and parameters was 4.1%, ranging from 0.7 to 10.5% (depending on application and sample). **Conclusion:** The CS-2500 compares well to the CA-1500 and offers the benefits of state-of-the-art functionality and ease of use in mid-volume coagulation laboratories.
*Product availability varies by country.
Sysmex is a trademark of Sysmex Corporation.

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QIP-MS: A specific, sensitive, accurate, and quantitative alternative to electrophoresis for the identification of intact monoclonal immunoglobulins

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Background: The development of the serum free light chain assay at the turn of the century heralded a significant improvement in the identification and quantification of these important biomarkers. In contrast, for the last 3 decades identification of monoclonal intact immunoglobulins has had little innovation. Here for the first time we present performance data for Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS), a polyclonal antibody-based technology to identify and quantify intact immunoglobulins. **Methods:** Modified sheep polyclonal antibodies (anti-IgG, -IgA, -IgM, -κ and -λ) were covalently attached to blocked magnetic microparticles. The microparticles were incubated with serum, washed, and then treated with 20mM TCEP in 5% (v/v) acetic acid to reduce patient immunoglobulin heavy and light chain disulphide bonds. Mass spectra were generated on a Microflex LT smart matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) system. Specificity was assessed using normal polyclonal serum (IgG = 8.25g/L, IgA = 1.67g/L, IgM = 0.7g/L, total κ = 7.7g/L, total λ = 3.67g/L) and patient sera containing monoclonal immunoglobulins (IgGκ = 33.2g/L, IgGλ = 28.3g/L, IgAκ = 21.1g/L, IgAλ = 20.4g/L, IgMκ = 23.1g/L, IgMλ = 19.1g/L). Accelerated stability was assessed at 22°C over 12 weeks using normal human serum. LoB and LoD were determined by serial titrations of polyclonal human serum diluted in sheep serum and linearity was assessed by serial titrations of a known monoclonal Ig diluted in normal polyclonal serum. Sensitivity was compared to CZE and IFE. **Results:** Mass spectra were acquired in positive ion mode covering the m/z range of 10,000 to 30,000 which includes the singly charged (+1, m/z 23330 to 24650) and doubly charged (+2, m/z 11168 to 12401) ions. Polyclonal molecular mass distributions for the light chains from: IgG (median IgGκ/IgGλ ratio 2.3:1 (CV=4.4%)), IgA (IgAκ/IgAλ = 1:1 (CV=3.5%)) and IgM (IgMκ/IgMλ=1.5:1 (CV3.7%)), total kappa, and total lambda were observed. No other peaks were observed in the polyclonal light chain molecular mass distributions confirming the high specificity of the antibodies. Normal human sera assessed at 22°C/12 weeks gave reproducible intensities for the polyclonal molecular mass distributions without loss of activity (48 week 4°C equivalent stability). The LoD for monoclonal proteins diluted into sheep serum were: 0.7mg/L for IgG, 1.4mg/L for IgA, IgM and total kappa, and 0.17mg/L for total lambda. Serial dilution of known monoclonal immunoglobulins into normal polyclonal serum gave acceptable linearity (IgGκ = y = 1.1x + 0.23, IgGλ = y = 1.02x + 0.06, LoD 24mg/L; IgAκ = y = 1.01x + 0.2, IgAλ = y = 0.95x + 0.44, LoD 8mg/L; IgMκ = y = 1.21x + 0.42, IgMλ = y = 1.15x + 0.17, LoD 8mg/L). In a blind study QIP-MS had a greater sensitivity for the detection of monoclonal immunoglobulins than either serum (100x) or immunofixation (10x) electrophoresis. **Conclusion:** QIP-MS provides a highly reproducible, linear, and sensitive alternative to conventional electrophoresis. The ability to measure a unique molecular mass for any myeloma paraprotein offers an innovative addition for the identification and quantification of monoclonal immunoglobulins.

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Pediatric reference ranges for the cobas m 511 Integrated Hematology Analyzer

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Background: The **cobas m** 511 system is a novel slide-based automated hematology analyzer that performs a CBC, WBC differential, reticulocyte count, and nucleated RBC count using automated digital microscopy. This single-center study determined pediatric reference ranges for the 26 parameters measured by the system. **Methods:** Residual EDTA whole blood samples were obtained from 245 healthy subjects covering four age ranges (6-≤24 months, 2-≤6 years, 6-≤12 years, and, separately for males and females, 12-≤18 years). A hematologist reviewed the subjects' medical records to rule out the presence of pathologies or therapies known to affect blood cell counts. Samples were analyzed on the **cobas m** 511 system and reference ranges were calculated as the central 95% of values obtained for each parameter according to CLSI guideline EP28-A3c. **Results:** Reference ranges for each pediatric cohort for the 26 blood count parameters measured by the system are presented in **Table 1** (*Data for RDW, RDW-SD, MPV, %NRBC, %NEUT, %LYMPH, %MONO, %EO, %BASO, %RET, #RET, and HGB-RET not shown)*. **Conclusions:** The observed reference range for the 26 parameters analyzed on the **cobas m** 511 Hematology Analyzer, and the age and sex differences, are consistent with reference ranges determined for other automated hematology analyzers.

Table 1: Pediatric reference ranges

Parameter [Units]	6-≤24 months	2-≤6 years	6-≤12 years	12-≤18 years (females/males)
WBC [10^3/µL]	5.06-14.94	4.46-13.42	4.28-12.68	3.86-11.03/3.87-12.53
RBC [10^6/µL]	3.97-4.98	4.01-5.02	3.99-4.99	3.88-5.18/4.09-5.34
HGB [g/dL]	10.8-13.3	11.0-13.6	11.6-14.1	11.1-15.0/12.3-15.9
HCT [%]	31.7-38.3	32.7-40.1	33.5-42.1	33.4-44.0/36.6-47.7
MCV [fL]	72.4-86.0	72.5-87.5	71.6-94.6	71.9-95.4/79.7-96.5
MCH [pg]	23.9-29.3	23.2-29.9	24.0-32.0	24.8-31.9/26.5-31.7
MCHC [g/dL]	32.4-35.1	32.4-34.9	32.4-35.4	32.2-35.1/32.5-34.5
PLT [10^3/µL]	222-551	213-579	199-420	184-409/138-409
#NRBC [10^3/µL]	0.00-0.01	0.00-0.01	0.00-0.02	0.00-0.01/0.00-0.03
#NEUT [10^3/µL]	1.45-5.64	1.18-6.51	1.63-6.90	1.69-6.70/1.50-9.55
#LYMPH [10^3/µL]	2.68-9.85	2.27-6.86	1.20-5.82	1.40-3.40/1.27-3.24
#MONO [10^3/µL]	0.38-1.51	0.34-1.07	0.29-0.91	0.22-1.09/0.35-1.30
#EO [10^3/µL]	0.02-0.91	0.07-1.82	0.01-1.37	0.01-0.51/0.02-0.64
#BASO [10^3/µL]	0.00-0.16	0.00-0.12	0.00-0.13	0.00-0.12/0.01-0.12

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Performance Evaluation of Automated Urine Analyzers to enhance laboratory efficiencies for Urinary Tract Infection Diagnosis

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Background: Rapid and accurate identification of urinary tract infection (UTI) is one of the most important issues in both health-care facilities and community settings. Urine culture is "gold standard" until now, but time-consuming, labor-intensive, and costly. Newly developed automated urine analyzers are expected to help reduce unnecessary culture and provide prompt data to clinicians for determination of the treatment plan. **Methods:** Compared to urine culture, a total of 528 samples were analyzed using five automated urine analyzers: UF-5000 (Sysmex Corporation), UAS800 (Siemens Healthineers), Cobas® u701 (Roche Dignostics), Iris iQ®200SPRINT (Beckman Coulter) and URiSCAN® PlusScope (YD diagnostics). The criteria for UTI was defined if the inoculated plate yields more than 10^4 CFU/mL from all specimens, or more than 10^3 CFU/mL from specimens that were collected from patients with foley catheter or urinary symptoms. Performances of indicators - leukocyte esterase (LE), nitrite, white blood cell (WBC) and bac-

teria - were analyzed by single indicator alone or in different combinations. **Results:** By considering the indicators alone, diagnostic performances of bacteria were various according to each of urine analyzers compared with urine culture. The specificity and negative predictive value (NPV) were 97.7%, 56.0%, 68.8%, 95.7%, 97.7% and 92.3%, 90.1%, 90.4%, 90.3%, 89.4% for UF-5000, UAS800, Cobas® u701, Iris iQ®200SPRINT and URiSCAN® PlusScope, respectively. For LE, the specificity ranged from 65.1 to 72.0% and NPV ranged from 87.1 to 88.9%. And after considering the combinations, diagnostic performances were improved, but not satisfactory because negative predictive values (NPVs) were less than 95%. The combination of LE and nitrite showed similar NPV, ranging from 88.9 to 90.3%. And the combination of bacteria and WBC showed NPV from 91.2 to 94.0%. **Conclusion:** In this study, we evaluated diagnostic performances of recently introduced automated urine analyzers compared with conventional urine culture. There are still limitations for automated urine analyzer to replace conventional urine culture in some cases. But, this study is a statistical approach regarding the feasibility as a screening test for UTI. As sensitive and rapid diagnostic tools, urine sediment analyzers can be one of the important tools in the near future, and reduce unnecessary culture and give a guidance for clinicians to determine treatment plan.

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A simple and rapid LC-MS method for identification of hemoglobin variants

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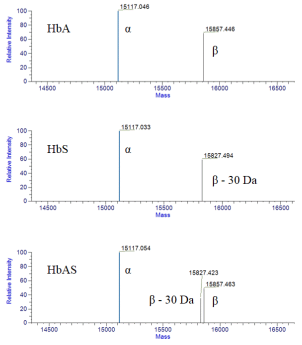
Background:
Hemoglobin (Hb), as the most abundant protein in the red blood cells, carries the important function of transporting oxygen from the lungs to tissues. Hb consists of four globin subunits (two α and two non-α units, e.g., β, γ and δ). Hemoglobinopathies are disorders resulted from gene mutations of the globin subunits which often cause structural change. Traditional methods of analyses are gel electrophoresis and chromatography; however, these methods are limited by low resolution and have difficulty in identifying less-commonly encountered variants. The goal of this work was to develop an LC-MS method to identify Hb variants that are challenging for the traditional methods. **Methods:** EDTA whole blood (10 µl) was mixed with 1 mL of sample buffer (3 % acetonitrile, 0.5 % formic acid and 1 % trifluoroacetic acid in water) followed by centrifugation at 13K rpm for 10 min. Ten microliter of the supernatant was then taken and diluted with sample buffer for another 100 folds. Samples were injected directly to a TLX-II LC system coupled with a Q-Exactive high resolution MS (Thermo Scientific). The intact Hb proteins were separated on a C18 column with total LC time of 5.5 min. Mass deconvolution was achieved using BioPharma Finder software (Thermo Scientific). **Results:** This LC-MS method was proven to be able to identify normal Hb and variants that were tested in both homozygous and heterozygous specimens (Figure 1). **Conclusion:** The developed LC-MS method is able to accurately identify Hb variants based on accurate mass of the intact protein subunits.
Figure 1. Deconvoluted mass of samples with HbA, HbS, and HbAS



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Significance of High Serum Ferritin in Discrimination of Various Diseases

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Background: Serum ferritin is an acute phase reactant and increases in various infectious and inflammatory conditions. Besides, serum ferritin is high in patients with transfusional iron overload. Severity of the rise in serum ferritin may be used as an indicative for much severe conditions, such as primary hemophagocytic syndrome. The objective of this study was to determine whether serum ferritin levels could be used to make a differential diagnosis between different diseases. **Materials and methods:** The patients below 18 years of age were included. Out of 11809 patients, who were tested for serum ferritin levels between January 2015 and February 2018, 260 (2.2%) were found to be above 1000 ng/ml. Those patients who were found to have serum ferritin above 1000 ng/ml were analyzed retrospectively in terms of the underlying diagnosis. **Results:** Mean age of the patients in study group were 90±71 months (0-215), 56% were males. Out of 260 patients, 70 (27%) had an underlying malignancy. Of the patients with malignancies, 32 (46%) had acute leukemia and 17 (24%) had adrenal neoplasm. Sixty-four patients (24.6%) had underlying infection, 21 (8%) had immune deficiency and infection, 17 (6.5%) had thalassemia, 17 (6.5%) had secondary hemophagocytic lymphohistiocytosis (HLH), 16 (6.1%) rheumatological diseases, 15 (5.7%) had chronic renal failure, 9 (3.4%) had secondary HLH, 9 (3.4%) had other types of hemolytic anemias, 6 (2.3%) had DBA, 6 (2.3%) had aplastic anemia, 2 osteopetrosis, 2 metabolc disease, 1 hydrops fetalis, 1 acute renal failure, 1 Celiac disease, 1 sickle cell disease, 1 cirrhosis, 1 had burn injury. The cut-off serum ferritin level that differantiates primary HLH from secondary HLH was 3282 ng/ml (100% sensitivity, 53%specificity). **Discussion:** High serum ferritin levels can be seen at diagnosis or during follow-up of various conditions. Levels above 3000 ng/ml might be indicative for primary HLH.

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Significance of Leukocyte VCS (Volume, Conductivity and Scatter) Parameters in the Diagnosis of Acute Exacerbations of Cystic Fibrosis

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Background: Cystic fibrosis (CF) is the most common inherited disease and is characterized by chronic sinopulmonary infection besides gastrointestinal, nutritional, and other abnormalities. The main clinical features of CF lung disease are chronic airway infection and repeated acute exacerbations, with a distinctive bacterial flora facilitating irreversible lung damage. Early diagnosis of acute exacerbations in CF patients is very important for the initiation of treatment. Our aim was to evaluate diagnostic significance of volume, conductivity, and scatter (VCS) parameters in cystic fibrosis with or without acute lung exacerbation, and healthy controls. **Methods:** CF patients (n=43) that are followed by the Department of Pediatric Respiratory Disease at Marmara University Pendik E&R Hospital and who were younger than 25 years of age were enrolled in the study. Acute exacerbations of CF cases were determined as episodes of acute worsening of respiratory symptoms and decline in lung function. A further age and gender matched 39 children without CF were also included as the control group. We extracted complete blood count data with VCS parameters of CF patients at the time of acute exacerbations which was determined clinically and during the follow-up visits without acute exacerbation together with those of the control cases from laboratory database. All parameters had been measured by Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). Data analysis was performed using SPSS Statistics 17 software. Results were expressed as the mean±standard deviation (SD). Comparisons among mean values of the groups were performed by ANOVA and $P<0.05$ was determined as significant.**Results:** The mean age was 7.4±7.6 years in CF group, while control group mean age was 5.8±4.5 years. WBC counts were significantly different in all groups. Mean neutrophil volume (MN-V-NE) (148±10, 148±8.6 respectively) and SD of neutrophil low-angle light scatter (SD-LALS-NE) (37.5±9.7, 35±9.5 respectively) were similar in CF with or without acute exacerbation ($P>0.05$). However, these parameters were significantly different compared to the control group (141±5.4; 30±6.5, respectively) ($P=0.002$; $P=0.022$, respectively). Lymphocyte median-angle light scatter (MN-MALS-LY) and upper median-angle light scatter (MN-UMALS-LY), and MN-MALS-MO of monocytes

were significantly increased in the CF group with acute exacerbation when compared with CF cases without acute exacerbation (P=0.006; P=0.002; P<0.001, respectively) but both groups of CF did not differ from control group (P>0.05). Mean conductivity (MN-C) of monocytes were significantly different among all groups (P<0.001, P<0.044, P<0.023). ROC curve analysis revealed 72.1% sensitivity and 69.8% specificity at 11 arbitrary units for SD of low-angle light scatter of lymphocyte (SD-LALS-LY) in the discrimination of acute exacerbation of CF patients. **Conclusion:** Volume, conductivity and scatter parameters like MN-MALS-LY, MN-UMALS-LY, SD-LALS-LY and MN-MALS-MO from automated analysers have emerged as promising parameters for the follow-up and management of acute exacerbation of cystic fibrosis. However, further studies are required to demonstrate their diagnostic value.

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Comparison of Five Automated Urine Sediment Analyzers to Manual Microscopy for Accurate Detection of Urine Particles

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Background: Urinalysis is one of the most commonly performed diagnostic test in clinical laboratory, and gives information regarding the wide range of disorders including kidney, metabolic, and systemic disease. For urine sediment analysis, microscopic examination is still "gold standard", but is time-consuming, labor intensive, and has large interobserver variation. Automation in urinalysis has been developed over time. And in this study, we evaluated analytical and diagnostic performances of recently introduced automated urine sediment analyzers. **Methods:** A total of 1016 samples were analyzed using five automated urine sediment analyzers: UF-5000 (Sysmex Corporation), UAS800 (Siemens Healthineers), Cobas® u701 (Roche Dignostics), Iris iQ®200SPRINT (Beckman Coulter) and URiSCAN® PlusScope (YD diagnostics). UF-5000 is based on flow-cytometry based system, and others are based on digital image-based system. Manual microscopy using KOVA chamber (KOVA International Inc.) was performed as reference method. **Results:** All of the five urine sediment analyzers showed acceptable performances in precision, linearity, and carry-over study, according to manufacturer's instruction. For semi-quantitative parameters, these five urine sediment analyzers showed good concordance rates within 1 grade difference for semi-quantitative parameters compared with manual microscopy: 92.2-94.7% for red blood cell (RBC), 92.2-93.8% for white blood cell (WBC), and 96.9-99.0% for squamous epithelial cell (SQEP). And diagnostic performances for qualitative parameters were also evaluated. The sensitivity and specificity for crystal were 44.8%, 49.1%, 61.2%, 68.1%, 18.1%, and 99.8%, 97.9%, 95.1%, 90.4%, 96.7%, for UF-5000, UAS800, Cobas® u701, Iris iQ®200SPRINT and URiSCAN® PlusScope, respectively. And the sensitivity and specificity for pathologic cast were 23.6%, 81.8%, 74.5% and 95.9%, 84.8%, 90.2%, for UF-5000, UAS800 and Cobas® u701, respectively. **Conclusion:** In this study, we compared five recently introduced automated urine analyzers with manual microscopy, for accurate detection of urine sediments. Compared to manual microscopy, there are still limitations in terms of detection of particles, particle recognition software, and interpretation system, and more technical advances are needed. But, automated urine analyzers are expected to reduce the burden of manual processing, with reliable results. And with image and microscopic review, routine urinalysis laboratories can provide more accurate results.

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Using QIP-MS to distinguish a therapeutic mAb from an endogenous M-protein in patients being treated for multiple myeloma

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Background: Therapeutic monoclonal antibodies (t-mAbs) daratumumab and elotuzumab have revolutionized the treatment of patients with multiple myeloma (MM). These t-mAbs present a challenge to laboratories using electrophoresis to monitor MM patients due to peak serum concentrations which can interfere with serum protein electrophoresis and immunofixation electrophoresis (IFE). Since the majority of monoclonal therapeutics have an IgG heavy chain isotype and a kappa light chain isotype, it is likely that newly developed t-mAbs will interfere with electrophoretic methods. An alternative solution is to utilize molecular mass to distinguish between an M-protein and a t-mAb. In this study we present the use of quantitative immunoprecipitation mass spectrometry (QIP-MS) as a method to distinguish patients Mprotein from daratumumab and elotuzumab. **Methods:** Briefly, modified sheep polyclonal antibodies (anti-IgG) were covalently attached to blocked magnetic microparticles. An IgGk patient monoclonal protein was diluted to 2.0g/L in normal human sera (IgG = 8.25g/L, IgA = 1.67g/L, IgM = 0.7g/L, total κ = 7.7g/L, total λ = 3.67g/L) and either daratumumab or elotuzumab was added at between 0-5-5g/L. Separately, 2xGK and 2xGL MM patient sera were diluted to 1.0 g/L in pooled normal human serum containing either daratumumab or elotuzumab at a concentration of 0.2g/L. Microparticles were incubated for 15mins, before being washed and treated with 20mM TCEP in 5%(v/v) acetic acid to reduce immunoglobulin heavy and light chains. Mass spectra were generated on a microflex LT "smart" MALDI-TOF-MS system. IFE was performed in a certified clinical laboratory in accordance with the manufacturer's instructions. **Results:** QIP-MS mass spectra identified the monoclonal GK patient at 2g/L in the same mass spectrum with daratumumab and elotuzumab present between 0.5-5g/L. By contrast, IFE was only able to identify the t-mAb when present at >1g/L. In an expanded study, QIP-MS was able to distinguish the monoclonal light chains originating from the patient's M-protein and the t-mAb at therapeutically relevant concentrations (0.2g/L) in all samples analyzed. The +2 charge states were monitored for the monoclonal light chains from daratumumab (11,689.0 *m/z)* and elotuzumab (11,710.5 *m/z*) and were clearly distinct from the +2 charge states of the monoclonal light chains from the patients (GK1 = 11,752.9 *m/z*, GK2 = 11,730.8 *m/z*, GL = 11,378.8 *m/z*, GL2 = 11,271.5 *m/z*). **Conclusion:** Our findings show that by combining the specificity of polyclonal anti-IgG antibodies bound to magnetic particles with the mass resolution and mass measurement accuracy of a MALDI-TOF mass spectrometer, daratumumab and elotuzumab were easily distinguishable from the patient M-protein, even in the presence of a high polyclonal background and at levels below the detection limit of IFE. Furthermore, this approach is agnostic to the therapeutic antibody and therefore can be used to monitor patients irrespective of their treatment modality, a distinct advantage over idiotypic gel shift assays.

Tuesday, July 31, 2018

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

Immunology

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Algorithm to avoid delay in the diagnosis of multiple myeloma in patients with incidental clinical findings at emergency service

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Background: The presence of incidental clinical findings (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia, acute kidney injury) related to Multiple Myeloma (MM) in Emergency Service and Primary Care should be studied for screening the existence of a possible MM. A quick panel based on serum protein electrophoresis (SPE) and quantification of serum free light chains (sFLC) enables sensitive quantification of monoclonal component in the study of MM. The application of this screening panel in patients with these incidental clinical finding without other diagnosis can help us to efficiently detect a possible MM in much sorter times. **Methods:** we studied three patients admitted to Emergency Service where we found incidental clinical finding characteristic of multiple myeloma (anemia, hyperproteinemia, intense bone pain). Sera of the three patients were sent to the Laboratory of Immunology for the screening of a monoclonal gammopathy. SPE were performed on CAPILLARYS 2 (Sebia) and the sFLC were measured by FREELITE (The Binding Site) turbidimetric assay. **Results:**
Case 1 (Man, 68 years)
Clinical finding: macrocytic anemia (9.0 g/dl hemoglobin), rouleaux formation of erythrocytes, discrete pancytopenia.
Protocol SPE+sFLC: weak peak in SPE (0.10 g/dl), sFLC ratio very altered (free kappa=14450 mg/l, free lambda=4.9 mg/l, ratio=2949) and immunoparesis.
Diagnosis: Light Chain Kappa Multiple Myeloma Stage 3 ISS
Case 2 (Woman, 65 years)
Clinical finding: hyperproteinemia (12 g/dl), hyperviscosity and thrombocytopenia.
Protocol SPE+sFLC: large peak (3.28 g/dl), altered sFLC ratio (free kappa=617 mg/l, free lambda=11.1 mg/l, ratio=55.59)
Diagnosis: Multiple Myeloma IgG Kappa Stage 2 ISS
Case 3 (Woman, 64 years)
Clinical finding: intense back pain
Protocol SPE+sFLC: large peak (3.22 g/dl), altered sFLC ratio (free kappa=3.15 mg/l, free lambda=102 mg/l, ratio=0.031)
Diagnosis: Multiple Myeloma IgA Lambda Stage 3 ISS
Conclusions: In the context of clinical symptoms (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia) that are alerts to suspect multiple myeloma it is advisable to apply the protocol (SPE+sFLC) for the screening of monoclonal gammopathies in patients without obvious clinical diagnosis. The combination of SPE and sFLC yields a fast and highly sensitivity approach in the screening of monoclonal gammopathies.

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HEK293-expresed GAD65 complements immunoblot for the comprehensive confirmation of anti-neural antibodies

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Background: Autoantibodies against the 65-kDa isoform of glutamic acid decarboxylase (GAD65) are the most prevalent markers of stiffperson syndrome (SPS) and also appear in progressive encephalomyelitis with rigidity and myoclonus (PERM), encephalitis and epilepsy. Standard method to determine anti-GAD65 is indirect immunofluorescence (IIF) on neuronal and pancreatic tissue sections. We present an effective full-length GAD65 antigen in an immunoblot format for specific detection of SPS and PERM-associated antibodies. **Methods:** 44 patients with clinically characterized SPS, 25 with PERM, 6 with paroxysmal or truncal dystonia/dyskinesia and 6 with hyperekplexia as well as 50 healthy controls were screened with an immunoblot providing several recombinant antigens (amphiphysin, CV2/CRMP5, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, zic4, Tr (DNER) and including full-length GAD65 expressed in eukaryotic cells) to identify the corresponding autoantibodies associated with paraneoplastic neurological autoimmune diseases. Anti-GAD65 positive results were verified by IIF using cerebellar and pancreatic tissue sections and GAD65-expressing HEK293 cells. **Results:** Antibodies against GAD65 were found in 24 patients with SPS (54.5 %), 19 with PERM (76.0 %, 1 also positive for anti-titin) and 1 with dystonia/dyskinesia (16.7 %). All cases were confirmed by IIF. Additionally, anti-amphiphysin autoantibodies were detected in 1 PERM (4 %) and 1 SPS (2.3 %) patient. Healthy controls did not reveal GAD reactivity. **Conclusion:** The multiparametric immunoblot used in this study allows comprehensive determination of antibodies against linear neurological antigens including anti-GAD65 to diagnose autoimmune movement disorders, in particular SPS and PERM.

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Serum concentrations of IgG subclass in the China adult population: relationship with age,gender, and atopy

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Objective: To explore the relationship between age, gender or lifestyle factors and serum concentrations of IgG subclass in China adults. **Methods:** One hundred and seventy healthy checkups were selected from the physical examination population, and the levels of serum IgG1, IgG2, IgG3 and IgG4 were measured using the immunonephelometric assay. Age groups were divided into five groups: 18 to 30 years old (34 cases), 31 to 40 years old (37 cases), 41 to 50 years old (38 cases), 51 to 60 years old (33 cases), more than 61 years old (28 cases). Smoking groups were divided into four groups: non-smoking group, mild smoking group (tobacco consumption of <5 cigarettes/day), moderate smoking group (tobacco consumption of 5-15 cigarettes/day), heavy smoking Group (tobacco consumption of >15 cigarettes/day). Drinking groups were divided into four groups: non-drinking group, mild drinking group (alcohol consumption of< 100g/week), moderate drinking group (alcohol consumption of 100-200g/week), severe drinking group (alcohol consumption of >200g/week). Metabolic syndrome group is divided into metabolic syndrome group and non-metabolic syndrome group, of which no metabolic syndrome group was divided into low-risk group and high-risk group. **Results:** The distributions of serum IgG1, IgG2 and IgG3 were approximately normal distribution in the population, but IgG4 was non-normal distribution. The median of serum IgG1, IgG2, IgG3 and IgG4 were 7.53g/L, 4.13 g/L, 0.51 g/L and 0.66 g/L. The median of serum IgG1/IgG, IgG2/IgG, IgG3/IgG and IgG4/IgG were 61.33%, 33.53%, 4.15% and 5.5%. ①The relationship between serum IgG subtypes and gender: The Serum IgG3 concentrations and the IgG3/IgG ratio had significant difference between male group and female group(P<0.01),but no significant differences in in IgG1, IgG2, and IgG4. ②The relationship between serum IgG subtypes and age: The serum IgG3 concentration in the 41-50 years old group was significantly higher than that in the 18-30 years old group(P<0.05). There was no significant difference on serum IgG1, IgG2 and IgG4 concentrations among all age groups. ③The relationship between serum IgG subtypes and smoking, drinking: The effect of smoking on the concentration of serum IgG1 and the IgG4/IgG ratio was significant. The levels of serum IgG1 and IgG3 in moderate drinking group were lower than those in non-drinking group (P <0.05). The IgG3 / IgG ratio in severe drinking group was lower than that of non-drinking group (P <0.05). ④The effect of metabolic syndrome on serum IgG subclass concentrations was not statistically significant. There were statistically significant differences on serum IgG3 and IgG3/IgG between high-risk group and low-risk group (P <0.05). **Conclusion:** IgG subtypes were studied in the general population for the first time in the domestic. The effect of gender and age on the concentration of serum IgG3 was significant, but there was no significant effect on serum IgG1, IgG2 and IgG4 concentrations. Serum IgG1 concentration was significantly correlated with smoking. The effect of drinking on serum IgG1 and IgG3 concentrations were statistically significant. Obesity and metabolic syndrome were not correlated with serum IgG subclass concentrations. The study provide some experimental evidence for the reference interval establishment of IgG subclass.

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Paediatric reference intervals for total IgG and IgG subclass concentrations on the Optilite® automated turbidimetric analyser

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Background: Total serum IgG consists of four structurally similar IgG subclasses (IgGSc); IgG1-4. Concentrations of IgGSc's vary with age, most noticeably in paediatric populations as the immune system matures. The measurement of IgG and IgGSc concentrations can aid in the identification of certain immune system disorders and immunodeficiencies. Determination of age-specific normal reference intervals for IgG and IgGSc antibodies is therefore essential to aid assessment of normal and abnormal concentrations. In the present study we report paediatric reference intervals for IgG and IgGSc concentrations on the Optilite™ turbidimetric analyser. **Methods:** Healthy controls were selected at Hospital Universitari Vall d'Hebron, Barcelona, Catalonia, Spain (n=145, median age 8 years, range 0-18). Patients with immune-mediated disorders or clinical syndromes were excluded. The study was approved by the Ethics Committee of Hospital Universitari Vall d'Hebron, Barcelona, Spain (PR_AG_134-2011). IgG and IgGSc concentrations were measured on an Optilite turbidimetric analyser (The Binding Site Group Ltd, Birmingham, UK). **Results:** Median total IgG and IgGSc concentrations for age groups 0-2, 3-4, 5-9, 10-14 and 15-18 years are provided in Table 1. The trend of concentrations across all age groups was IgG1>IgG2>IgG3>IgG4. Significant increases in concentration were observed for total IgG, IgG2 and IgG4 between the 0-2, 5-9 and 10-14 years age groups ($p<0.05$). There was a strong correlation between the total IgG concentration and summation of the IgGSc concentrations ($R^2=0.89$, $p<0.0001$, $y=0.98x+14.51$). **Conclusion:** We have generated age-specific reference intervals in healthy children for total IgG and IgGSc measurements using the Optilite turbidimetric analyser. These intervals will help identify individuals with abnormal concentrations and thus will aid in the diagnosis of both primary and secondary immunological disorders.

Median IgG and IgGSc concentrations in a healthy paediatric population. Concentrations in mg/dL.

	Age (years)				
	0-2	3-4	5-9	10-14	15-18
IgG	849.8(n=22)	820.4(n=19)	943.8(n=39)	1045(n=40)	975.8(n=21)
IgG1	615.5(n=22)	538(n=19)	653(n=40)	640(n=42)	543.5(n=22)
IgG2	114.8(n=22)	130.3(n=19)	206.3(n=40)	268.6(n=42)	321.5(n=22)
IgG3	50.4(n=22)	59.3(n=19)	56.95(n=40)	77.25(n=42)	66.4(n=22)
IgG4	8.2(n=22)	8.3(n=19)	22.65(n=40)	47.05(n=42)	24.35(n=22)

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Distribution of *PTPN22* 1858CT (R620W) polymorphism in Mexican older adults with frailty syndrome and its relationship with clinical parameters

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Background: Frailty is a late-life syndrome of etiology unknown, characterized by muscle weakness, weight loss and fatigue. Even though the knowledge of the pathophysiological mechanisms underlaying frailty remains limited, evidence suggests that inflammation has a major role in the pathophysiology of frailty. Recent studies have been shown alterations in the activation and differentiation of $CD4^+$ T cells in older adults, changes made mainly in the different events of the T-cell receptor signaling cascade. The tyrosine phosphatase protein non-receptor 22 (*PTPN22*) gene encodes the protein tyrosine phosphatase lymphoid (LYP), an important molecule in the immune system and actively involved in the negative regulation of the T cell activation. So, based in these findings, we hypothesize that genetic variants within *PTPN22* gene could affected the normal function of LYP. **Objective:** To analyze the association of *PTPN22* 1858C>T (R620W) polymorphism with the frailty syndrome in Mexican older adults and its relationship with clinical parameters. **Methods:** One thousand twenty-four elderlies were interviewed, 743 accepted blood sampling, 114 were excluded by incomplete data for frailty. Frailty was defined as the presence of ≥3 of five components (weakness, slowness, lack of energy, weight loss, fatigue). Genomic DNA was extracted from the peripheral blood of all subjects (n=629). *PTPN22* 1858C>T (R620W) polymorphism was genotyped by Real Time PCR with pre-designed TaqMan Probes in the StepOne Plus kit. Statistical analysis was carried out using SPSS v20.0. **Results:** The average age 78 ± 6.0 years and 52.5% were women. Frailty subjects showed low mental state ($p<0.001$), greater disability for activities of daily living and instrumental ($p<0.001$) and low economic perception ($p<0.001$). Genotypic and allelic frequencies for the *PTPN22* 1858C>T (R620W) polymorphism did not show significant differences between study groups ($p>0.05$). Moreover, we evaluated the clinical characteristics according to each genotype in each group and we observed that the pre-frail subjects carrying the CT genotype had a higher percent of weight loss than the carriers of the CC genotype ($p=0.028$), whereas in the frail subjects, carriers of the CC genotype had a higher percent of low physical activity level than those with the CT genotype ($p=0.013$). **Conclusions :** Our results suggest that the *PTPN22* 1858C>T (R620W) polymorphism is not relevant in the genetic susceptibility for frailty syndrome in Mexican elderly. However, it was found that there is a significant and independent relationship between the polymorphism and weight loss and low physical activity level, two of the clinical components of the fragility phenotype.

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Abbott Alinity i System Sigma Metrics for Immunoassays

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Background: Assay performance is dependent on the accuracy and precision of a given method. These two attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for more than 12 immunoassays tested on the Alinity i system. In 2017, a separate and distinct subset of 13 immunoassays were analyzed and presented as an AACC poster using similar methods. **Methods:** A sigma metric was estimated for each assay and was plotted on a method decision chart. The sigma metric was calculated using the equation: sigma = (%TEa - |%bias|) / %CV. A precision study was conducted at Abbott on each assay using the Alinity i system per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, 100-200 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity i and ARCHITECT i2000SR systems. The 1st replicate from the Alinity i system was regressed versus the mean ARCHITECT i2000SR concentration and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated near a criticial concentration level. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity i system and the ARCHITECT i system, where the ARCHITECT i system within-laboratory %CV and mean concentration values were obtained from the assay package inserts. **Results:** The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near a criticial concentration level. The precision profile charts of the within-laboratory %CV results for the Alinity i system overlaid with the ARCHITECT i system showed similar performance across the subset of assays evaluated. **Conclusion:** Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity i system immunoassays had sigma metrics greater than 5. The precision performance on the Alinity i and ARCHITECT i systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

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Effects of resolvin D1 and E1 on systemic and hepatic inflammation caused by ethanol and LPS challenge: a time course analysis

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Background/Purpose: Alcoholic liver disease (ALD) is a major health problem in the US and worldwide. ALD encompasses a variety of liver pathologies, including steatosis, an early stage of the disease, and steatohepatitis, a more progressive stage with both hepatic and systemic inflammation. Currently, there is no FDA-approved therapy for any stage of ALD. Resolvins are lipid metabolites derived from ω3-polyunsaturated fatty acids, and exhibit anti-inflammatory and pro-resolution effects in several pathological conditions. Herein, we tested the hypothesis that resolvins will attenuate liver injury caused by ethanol (EtOH) and lipopolysaccharide (LPS) administration via reduction of systemic and hepatic inflammation in an animal model of ALD**. Methods:** C57BL6/J male mice received EtOH by oral gavage (EtOH-treated mice) or an isocaloric/isovolumetric maltose-dextrin solution (control mice) for 3 consecutive days. LPS was administered 24h following the final EtOH gavage along with either resolvin E1 (RvE1) or D1 (RvD1). Animals were euthanized 4h or 24h later. Liver injury was evaluated by plasma ALT activity. Hepatic neutrophil infiltration was evaluated by chloroacetate-esterase staining. Plasma cytokines/chemokines were measured by the multiplex magnetic bead-based Luminex immunoassay. Hepatic pro-inflammatory gene expression and inflammasome activation were determined by qRT-PCR. **Results**: Compared to plasma ALT levels in control mice (16+0.8 U/L), EtOH and LPS administration significantly increased ALT levels at 4h (74.71 ± 6.03 U/L), and to a greater magnitude at 24h post-LPS (305 ± 64 U/L). EtOH+LPS treatment synergistically increased plasma ALT as compared to EtOH alone (~12-fold) or LPS alone (~5-fold). Importantly, the EtOH+LPS-induced plasma ALT at 24h was significantly attenuated by RvD1 (141 ± 49 U/L), and to a lesser extent by RvE1 (209 ± 49.3 U/L). A Luminex immunoassay revealed that the EtOH+LPS-mediated induction of plasma pro-inflammatory mediators, including TNF-α, IL-6, LIX, CXCL-1, MCP-1 and MIP-2, peaked at 4h, but remained elevated at 24h as compared to control animals. Notably, TNF-α was significantly decreased by both RvE1 and RvD1 at 24h, and a similar trend was observed at 4h. Further, compared to control animals, mice treated with EtOH+LPS had markedly increased hepatic neutrophil infiltration at both 4h and 24h, which was attenuated by RvE1 and RvD1 treatment. EtOH+LPS significantly induced expression of the hepatic neutrophil chemoattractants, *Cxcl-1* and *Cxcl-2,* and pro-inflammatory cytokines, *Tnf-α*, *Il-6*, *Il-1β, Il-18* at both 4h and 24h. Notably, RvD1, but not RvE1, significantly attenuated the EtOH-LPS-mediated *Cxcl-1*, *Cxcl-2*, *Il-1β* and *Il-18* induction at 24h, suggesting distinct inflammatory pathway-specific actions of resolvins. In addition, RvD1 attenuated expression of the inflammasome components *Caspase-1*, and *Asc*, suggesting a suppressive effect of RvD1 on inflammasome activation. **Conclusions:** Collectively, our data demonstrate that circulating and hepatic markers of inflammation were elevated in a time-dependent manner in liver injury induced by EtOH and LPS, with the peak inflammation observed at 4h compared to 24h post-LPS injection. Importantly, resolvin treatment significantly ameliorated inflammatory responses observed at 24h, and to a lesser extent at 4h, suggesting a potential pro-resolution effect, and thus may be a promising novel therapy for ALD.

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Performance Evaluation of Anti-tTG IgA Assay on the Fully Automated BioCLIA®1200 Immunoassay Analyzer

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Background: Celiac disease (CD) is a life-long condition in which consumption of dietary gluten, leads to chronic inflammation and damage of the small intestinal mucosa. Tissue transglutaminase (tTG) has been identified as the major autoantigen in CD. IgA antibodies against tTG are highly disease specific serological markers for CD. Recently, the anti-tTG IgA assay coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system has been evaluated and launched. **Methods:** In this study, the analytical performances including dilution linearity, limit of detection (LOD), precision (intra-assay & inter-assay) of BioCLIA® anti-tTG IgA kit were evaluated, according to the CLSI guidelines. Total of 200 clinical samples, including 100 each of positive and negative samples from France, were both evaluated with a commercial anti-tTG IgA FEIA and BioCLIA® anti-tTG IgA kit. Furthermore, the various type of clinical samples including celiac disease (CD, N=10), chronic diarrhea (N=130), autoimmune thyroid disease (N=50), type 1 diabetes (T1DM, N=50), rheumatoid arthritis (RA; N=50) and healthy donors (N=150) collected from local Chinese hospitals were also evaluated and analyzed. **Results:** The performance evaluation of HOB BioCLIA® anti-tTG IgA kit showed the accurate and faster results with an extended working range and good reproducibility. Compared with the FEIA kit, we observed the comparable sensitivity (99%; N=99/100) and specificity (98%; N=98/100) between two kits. The clinical sensitivity of CD were 90% (N=9/10), while the specificity for Chronic Diarrhea, Autoimmune Thyroid Disease, T1DM, RA, and healthy donors were 99.2% (N=129/130), 96% (N=48/50), 94% (N=47/50), 100% (N=50/50) and 99.3% (N=149/150), respectively. **Conclusion:** The anti-tTG IgA assay on the BioCLIA® 1200 automated platform exhibits an excellent sensitivity, a wider measurable range and shorter reaction time compared with traditional ELISA. It serves as a promising and environmental-friendly alternative for FEIA assay in the detection of CD autoantibodies.

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New serological markers for celiac disease: Anti-neo-epitope human and microbial transglutaminases antibodies

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Objectives: Microbial transglutaminase (mTg) and human tissue Tg (tTg) complexed to gliadin peptides present neo-epitopes. Antibodies against these complexes are called tTg neo-epitope and mTg neo-epitope. Reliability of antibodies against the non-complexed and complexed forms of both transglutaminases to reflect intestinal damage and to diagnose the pediatric Celiac Disease (PCD) was compared. **Methods:** 95 PCD patients, 99 normal children (NC) and 79 normal adults (NA) were tested using the following ELISAs detecting IgA, IgG or both IgA+IgG combined: tTg (for in house research use only), *AESKULISA*® tTg New Generation (tTg neo-epitope (tTg-neo)), *AESKULISA*® mTg (RUO) and *AESKULISA*® mTg neo-epitope (mTg-neo, RUO). Revised Marsh criteria were used for the degree of intestinal injury. **Results:** All anti-mTg-neo and anti-tTg-neo levels were higher ($p<0.001$) compared to the single antigens. tTg-neo IgA and IgG+IgA were higher than mTg-neo IgA and IgA+IgG ($p<0.0001$). The antibody activities reflecting best the increased intestinal damage were: mTg-neo IgA > mTg-neo IgA+IgG > tTg-neo IgG ≥ mTg-neo IgG > tTg-neo IgA > tTg-neo IgA+IgG. Taken together, mTg-neo IgG and tTg-neo IgA and IgA+IgG correlated best with intestinal pathology ($r=0.5633$, $r=0.6165$ & $r= 0.6492$; $p<0.0001$, $p<0.0001$ and $p<0.0001$, respectively). **Conclusion:** The complexed forms of both transglutaminases exhibited a higher OD activity and better reflected intestinal damage in PCD, compared to the non-complexed forms. mTg is immunogenic in children with coeliac disease and by complexing to gliadin its immunogenicity and intestinal pathology reflection is enhanced.

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Method comparison of AESKUSLIDES ANCA for the diagnosis of ANCA-associated Vasculitis

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Background: AESKUSLIDES ANCA is an indirect immunofluorescence assay used to detect anti-neutrophil cytoplasmic autoantibodies (ANCA) in human serum. This in vitro diagnostic assay is used as an aid for the diagnosis of ANCA-associated vasculitis (AAV) in conjunction with other clinical and laboratory findings. Methods: A method comparison of ethanol and formalin fixed granulocytes was carried out between AESKUSLIDES ANCA (AESKU.Diagnostics) and the NOVA Lite ANCA of INOVA. 507 clinical serum samples (comprising 135 serum samples from patients with AAV and 375 samples from patients with other diseases) were analyzed by standard IFA protocols. Results were obtained by manual processing and reading. Results: In this cohort, AESKUSLIDES ANCA Ethanol slides show higher sensitivities (48.5% vs. 36.4%) and specificities (69.3% vs. 55.2%) compared to INOVA. AESKUSLIDES ANCA Formalin slides show higher sensitivities (50.0% vs. 37.9%) and similar specificities (90.7% vs 91.5%) compared to INOVA. Conclusions: AESKUSLIDES ANCA Ethanol showed higher diagnostic sensitivity (48.5%) and specificity (69.3%) compared to the predicate assay NOVA Lite provided by INOVA (36.4%, 55.2%). This is due to the fact, that AESKU assay detects more positives in the AAV cohort, and less positives in the other disease groups. AESKUSLIDES ANCA Formalin showed a diagnostic sensitivity (50.0%) compared to the predicate assay NOVA Lite provided by INOVA (37.9%). However, the diagnostic sensitivity was comparable between the two (90.7% vs 91.5%).

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The prevalence of ASCA IgA and IgG antibodies is increased in manic bipolar disordered patients

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Objectives and study: anti-Saccharomyces cerevisiae antibodies (ASCA) are frequent in gastrointestinal inflammatory diseases. Given the concept of gut-brain axis[1] and inflammation induced leaky gut syndrome in psychiatric settings, the prevalence of ASCA is of interest to be analyzed in bipolar disorder. **Aim:** To study ASCA prevalence in bipolar disorder patients. **Methods:** IgA+IgG (Check) ASCA were detected by ELISA (*AESKULISA®* Crohn's-Check), in 170 bipolar patients, (52 depression and 118 manic), and compared to 69 healthy controls. **Results:** We found that the prevalence of ASCA Check positivity was significantly higher in manic bipolar patients as compared to healthy controls (25% and 13% respectively p< 0.0023)**. Conclusions:** Increased prevalence of ASCA Check antibodies is found in manic phase of bipolar disorder. Environmental processed nutrients, enteric comorbidity or overall enhanced activated immune state can explain this high prevalence.

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Performance evaluation of the specific proteins panel on the Alinity c system

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Background: Specific proteins are valuable markers for a variety of diseases including microbial infections, inflammatory response, cardiac risk, and even cancer. Abbott provides a broad spectrum of specific protein assays which enable diagnosis and management of many immune system related diseases. These assays include testing levels of the complement system to monitor inflammatory response and to help diagnose and monitor autoimmune diseases, such as rheumatoid arthritis. The Alinity ci system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 1350 tests per hour. The Alinity ci system has an increased reagent load capacity, holding up to 70 Clinical Chemistry reagents, onboard QC and calibrators, clot and bubble detection ability, and smartwash technology to provide consistent and reliable results. **Objective:** To demonstrate the analytical performance of representative assays from the Specific Proteins Panel of the Alinity c system, which consists of assays that utilize photometric technology for the quantitative determination of analytes in human serum or plasma. **Methods**: Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison were assessed per Clinical and Laboratory Standards Institute (CLSI) protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met. **Results:** The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the Specific Proteins Panel are shown in the table below.

Assay	Total %CV	LoQ	Method Comparison to ARCHITECT (Slope/r)	Measuring Interval
Immuno-globulin A	≤ 1.8	3 mg/dL	0.98/1.00	5 mg/dL to 3850 mg/dL
Immuno-globulin G	≤ 1.5	10 mg/dL	0.99/1.00	320 mg/dL to 4675 mg/dL
Immuno-globulin M	≤ 3.2	3 mg/dL	1.02/1.00	5 mg/dL to 1815 mg/dL
Prealbumin	≤ 2.2	1.0 mg/dL	1.00/1.00	3 mg/dL to 66 mg/dL
Complement C3	≤ 1.8	5 mg/dL	1.03/1.00	11 mg/dL to 385 mg/dL
Complement C4	≤ 1.7	0.7 mg/dL	0.98/1.00	2.9 mg/dL to 72.0 mg/dL
Haptoglobin	≤ 1.5	3 mg/dL	0.99/1.00	8 mg/dL to 300 mg/dL
Apolipoprotein A1	≤ 1.4	3 mg/dL	1.02/1.00	16 to 310 mg/dL
Apolipoprotein B	≤ 3.7	3 mg/dL	0.98/1.00	11 to 240 mg/dL

Conclusion: Representative clinical chemistry assays utilizing photometric technology on the Alinity c system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT clinical chemistry assays.

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Laboratory biomarkers in the monitoring of a patient with multiple myeloma

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Background: Multiple Myeloma (MM) is a malignancy of B cells characterized by an atypical proliferation of plasm cells. IgD MM has a very low incidence (2% of MM cases) and it´s characterized by an aggressive course and a worse prognosis than other subtypes. The free light chains in serum (FLC) are very important markers for monitoring patients with multiple myeloma (MM) and other monoclonal gammopathies. When the serum FLCs are present in low concentrations, they are difficult for the detection by conventional methods as serum protein electrophoresis (SPE) and immunofixation (IFE). We report the case of a patient where FLCs are either undetectable or barely detectable using the conventional qualitative assays. **Case report**: A 50 years old man was diagnosed in June 2011 of IgD Kappa multiple myeloma with primary amyloidosis associated. He began treatment with VAD (*vincristine*, doxorubicin and dexamethasone) and hemodialysis. He received three cycles of VAD from July 2011 to August 2011 but the κ/λ FLC ratio was altered during this treatment (from an initial value of 1570 mg/L in July to a value of 1633 mg/L in August). The IFE was positive (IgD Kappa) during the treatment. Due to the minimum response of the disease and the development of demyelinating neuropathy, the treatment was changed to bortezomib and dexamethasone. Then, the patient received eight cycles from September 2011 to April 2012 with a normalization of the κ/λ FLC ratio from an initial value of 1579 mg/L in September to a value of 1.62 mg/L at the end of March 2012 with negative IFE. The patient´s condition improved with this treatment and achieved the complete remission (CR). Three months later, the κ/λ FLC ratio began to increase predicting a relapse with a value of 2.52 mg/L in July, 4.27 mg/L in August, 60.23 mg/L in October and a maximum value of 135.85 mg/L in December. In these months, the IFE was normal. In January 2013, the κ/λ FLC ratio remained altered (97.41 mg/L) and the IFE was positive (IgD Kappa) for first time in the relapse. **Conclusions**: This case is a good example of the utility k/λ FLC ratio in the monitoring of multiple myeloma. The k/λ FLC ratio can detect when the chemotherapy applied isn´t completely effective or it can predict future relapses in the patient.

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Laboratory biomarkers in the identification of residual disease in patients with multiple myeloma

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Background: The quantification of heavy/light chains pairs (HLC) by the new immunoassay Hevylite is based on the recognition of epitopes spanning the junction of the immunoglobulin´s heavy and light chains. This assay can identify separately the different light chain types of each immunoglobulin class: IgGK, IgGL, IgAK, IgAL, IgMK and IgMK. Of particular interest and novelty is the possibility to quantify separately both isotypes of the tumor related immunoglobulin. In this clinical case we show the utility of the quantification of HLC IgAK, IgAL and IgAK/IgAL ratio as a method to monitoring and identifying residual disease in a patient with IgA-Kappa Multiple Myeloma. **Case presentation:** We present the case of 51 years old man diagnosed of IgAK Multiple Myeloma ISS III stage [hypercalcemia (16.6 mg/dl), increased IgA (4449 mg/dl) and total proteins (12.6 g/dl), normocytic anemia (9.5 g/dl of hemoglobin), altered ratio of serum free light chains (free kappa=219 mg/dl, free lambda=1.01 mg/dl, ratio=216.83) and osteolytic bone lesions (punched-out lesions in skull and vertebral compression)]. At diagnosis (Day 0) the serum proteinogram (SPE) shows a well-defined monoclonal large peak in the gamma region (4.34 g/dl correspond to monoclonal component) identified by immunofixation as IgA-Kappa. The IgA HLC ratio (IgAK=66.604 g/l, IgAL=6.302 g/L, ratio=10.57) identified clonal disease IgA-Kappa at diagnosis. The patient began treatment with Bortezomib, Cyclophosphamide and Dexamethasone and the monoclonal protein was monitorized by SPE, IFE and HLC. During the treatment, the monoclonal protein was decreasing with reduction of the peak in SPE and the HLC ratio remained altered confirming the presence of the monoclonal protein. The monoclonal component IgA-Kappa was decreasing due to the good response to the treatment. At day +58 (after 4th cycle of chemotherapy) there was a little peak in SPE (0.18 g/dl of monoclonal component), with positive IFE and altered ratio HLC (IgAK=3.566 g/l, IgAL=0.664 g/l, ratio=5.37). At day +68 the SPE was negative but the HLC ratio remained altered (IgAK=3.566 g/l, IgAL=0.664 g/l and ratio=5.37) confirming the existence of monoclonal protein that it was verified by IFE. At day +131 (end of 5th cycle of chemotherapy) the SPE, HLC and IFE were negative confirming the absence of monoclonal protein due to the good response to the treatment. At the end of the treatment (day +184) after six cycles of chemotherapy the patient achieved a status of complete remission with negative immunofixation, <5% of plasma cells in bone marrow and normal HLC pairs and ratio. **Conclusion:** The monitorization of IgA MM requires the measures of SPEP, IFE and total IgA. The use of the HLC IgAK, IgAL and their ratio IgAK/IgAL presents itself as an alternative method with high sensitivity for monitoring these patients, particularly in situations where traditional techniques show limitations (e.g. low concentracions, interference of other serum proteins, strong polyclonal background). The high sensitivity of the determination of HLC allows typing monoclonal component providing equivalent information to the immunofixation, with the added value of reporting a quantitative value.

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Diagnostic Utility of Antitransglutaminase Antibodies IgA in the study of Celiac Disease

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Celiac disease (CD) is an autoimmune disease caused by a gluten proteins intolerance and manifested in individuals with genetic predisposition when there is an intake of foods rich in gluten such as wheat, barley or rye. This disease occurs with severe atrophy of the mucosa of the upper small intestine and the only treatment currently available consists of a gluten-free diet that must be strictly maintained. Currently, in clinical laboratories, the study of CD is usually initiated with the determination in serum of anti-tissue transglutaminase IgA antibodies (t-TGIgA) and / or IgG (t-TGIgG). These techniques present a series of advantages such as complete automation and high sensitivity and specificity, which have displaced other serological tests such as anti-endomysial antibodies and anti-gliadin antibodies as the technique of choice. However, a diagnosis of celiac disease should not be based only on a positive result of anti t-TGIgA but should be based on the evaluation of the set of clinical and laboratory data available, mainly, the small bowel biopsy that remains the test "gold standard" for the diagnosis of CD. The main purpose of this study was to analyze the results of t-TGIgA antibodies in our laboratory and compare them with the diagnoses of patients to check how many of those positive results of anti t-TGIgA were confirmed or not with a diagnosis of CD. The samples used were serum from patients who the t-TGIgA antibodies was requested for a period of 8 months in 2017. Samples were processed in the ZENIT RA autoanalyzer (A.MENARINI diagnostics) by chemiluminescent immunoassay (CLIA). A value greater than 10 AU/mL was considered a positive result. Next, we reviewed the clinical histories of those patients whose results were positive to verify the definitive diagnoses. In this period of time, a total of 3040 determinations of anti-TGIgA antibodies were made by CLIA, of which 74 (2,4%) were positive. Of these 74 patients, 50 (67,6%) were diagnosed with CD, 10 patients (13,5%) with probable diagnosis/suspicion of CD, and 14 (18,9%) who were not diagnosed with CD. Definitely, anti-TGIgA antibodies have a high sensitivity for screening in the study of CD because in this study, of the total number of patients with positive results, 81,1% were confirmed with a definitive or probable diagnosis of CD.

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Analysis of the Chemiluminiscent Immunoassay as a method of screening in the study of Systemic Autoinmune diseases

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Systemic autoimmune diseases (EAS) are a group of diseases with extensive clinical expression, chronic course, among which are systemic lupus erythematosus (SLE), polymyositis / dermatomyositis (PM / DM), mixed connective tissue disease (EMTC), Sjögren's syndrome (SS) or systemic sclerosis (ES). In the study of EAS, the determination in serum of antinuclear antibodies (ANA) is usually used as the initial screening test. ANA are a heterogeneous group of antibodies developed by the immune system directed against a large variety of antigens located in the nucleus and cytoplasm of cells of different organs and body tissues. In our laboratory, the determination of ANA is carried out using the chemiluminescence technique (CLIA). However, due to the false positives presented by this method, when a positive result is obtained by CLIA, confirmation by indirect immunofluorescence (IFI) on human epithelial (HEp-2) cells is required, which is the "gold standard" test. However, the IFI has disadvantages that are mainly its subjectivity and a higher cost. The aim of this study was to calculate the positive predictive value of the CLIA technique, comparing it with the IFI to evaluate its usefulness in the screening of EAS. The results obtained during the second semester of 2017 were collected in serum from patients who were asked to determine ANA. Samples were processed by CLIA in the LIAISON® Analyzer (Diasorin Palex Medical). A sample with a value equal to or greater than the 1.5 index was considered a positive result. In total, 2,875 samples were processed, of which a positive value was obtained in 312 (10,9% of the total). These 312 samples were analyzed by IFI using the Olympus CX41 fluorescence microscope. Titrations with IFIs less than 1:40 were considered negative and those greater than or equal to 1:40 were positive. A total of 198 positive results (63,5%) and 114 negative results (36,5%) were obtained by IFI. In the positive results there were titrations from 1/40 to 1/1280 and with the following distribution of patterns:

- SPECKLED: 64
- RETICULAR/MITOCHONDRION-LIKE:32
- CENTRÓMERE:32
- HOMOGENEOUS:20
- CYTOPLASMIC:20
- NUCLEOLAR:10
- CYTOPLASMIC AND SPECKLED:4
- CYTOPLASMIC AND NUCLEAR DOTS:4
- CYTOPLASMIC AND NUCLEOLAR:2
- NUCLEAR DOTS:2
- HOMOGENEOUS AND SPECKLED:2
- P.C.N.A:2
- NUCLEOLAR AND NUCLEAR DOTS:2
- RETICULAR/MITOCHONDRION-LIKE AND NUCLEAR DOTS:2

With these results, the positive predictive value of the CLIA technique obtained was 63.5%. This confirmed one of the disadvantages of chemiluminescence in the initial screening of EAS, which was the presence of false positives. In conclusion, the CLIA technique is useful as a method of scrutiny in the study of EAS since it is an automated, objective and highly sensitive technique. However, before a positive result this must be confirmed by IFI.

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Necrotizing myopathy caused by combination of antisynthetase syndrome and mycobacterial infection non detected by Quantiferon: Diagnosis by laboratory methods.

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Background: Antisynthetase syndrome is an inflammatory muscle disease related to dermatomyositis and polymyositis. The hallmark of antisynthetase syndrome is the presence of serum autoantibodies directed against aminoacyl-tRNA synthetases. These are cellular enzymes involved in protein synthesis. Individuals with antisynthetase syndrome and other autoimmune disorders are also at increased risk of infection, both due to immune compromise from the disease itself and due to immunosuppressive therapy. Moreover, false negative or indeterminate Quantiferon results can be seen in elderly, immunocompromised, chronic and severely diseased patients. **Methods:** A 62 year old Afro-Caribbean woman with a past medical history of diabetes mellitus type II with recent travel to Trinidad arrived to our institution with fever and left upper extremity muscle pain. A prior muscle biopsy showed necrotizing myopathy, for which she had a 4-month course of steroids treatment. Chest CT scan demonstrated heterogeneous appearance of the left pectoral muscle with surrounding fluid attenuation, subcutaneous fat infiltration and scattered subcentimeter reactive lymph nodes. Relevant labs include bandemia, elevated procalcitonin, liver function tests (LFT) and creatine phosphokinase (CPK). An extensive work-up was done for autoimmune disease and myositis. Myositis panel assay, a test that detects serum markers for myositis using Western Blotting, which includes EJ antibody, RNP, Mi-2, Ku, Signal Recognition Particle (SRP), Mi-2, PL-7, PL-12, and OJ antibodies was performed. HTLV1 and 2 antibodies and Quantiferon test, were also performed. **Results:** Myositis panel assay was positive for EJ antibody, and negative for RNP, Mi-2, Ku, Signal Recognition Particle (SRP), Mi-2, PL-7, PL-12, and OJ antibodies. Multiplex flow immunoassay was negative for Proteinase-3, Myeloperoxidase, ANA, Scleroderma, B2-glycoprotein and anti-smith antibodies. Chemiluminescent immunoassay detected HTLV1 and 2 antibodies in this patient, but the immunoblot was negative for the HTLV-1 and -2 virus. Quantiferon for tuberculosis was indeterminate. The patient decompensated and expired on the 9th day of the hospitalization. During the hospitalization, questions were raised as to whether the patient had an autoimmune myositis versus other causes of necrotizing myopathy. At autopsy, chronic myopathic pathology with muscle atrophy and fibrosis was seen. In addition, acute abscesses with areas of necrotizing inflammation were identified in chest wall, lymph node, adipose, muscle and liver tissue, showing numerous acid-fast bacilli on FITE stain. Interstitial lung disease, interstitial inflammation and fibrosis were also present. Positive mycobacterium tuberculosis complex was identified by molecular detection on Paraffin. **Conclusion:** Our case illustrates the challenging diagnostic process of Antisynthetase syndrome in a patient with concurrent mycobacterial infection with tropism for soft tissue. Furthermore, patients who are immunocompromised and have positive anti EJ antibodies may have unreliable Quantiferon test results. Alternative approaches should be sought for determining patient's mycobacteria status in these clinical settings.

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Performance Evaluation of Anti-Proteinase 3 IgG Antibody Assay on the BioCLIA® 1200 Automated Chemiluminescence Immunoassay Analyzer

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Background: Anti-neutrophil cytoplasmic antibody (ANCA) testing has revolutionized the diagnosis and treatment of the various autoimmune mediated vasculitis. The pANCA and cANCA auto-antibodies have proven to be useful clinically and interesting scientifically for the detection of diseases such as Wegener's granulomatosis (WG), crescentic glomerulonephritis, polyarteritis nodosa and Churg-Strauss syndrome. Anti-proteinase 3 (PR3) IgG antibodies is one of the primary cANCA present in patients with WG and the specificity of anti-PR3 antibodies for WG was determined to be 97% Recently, the innovative HOB BioCLIA® anti-PR3 assay, coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system has been launched. **Methods:** In this study, the analytical characteristics including limit of detection (LOD), precision (intra-assay & inter-assay), dilution linearity, and interference were evaluated by HOB BioCLIA® anti-PR3 assay according to the CLSI guidelines. Furthermore, total of 100 clinical samples with indirect immunoflurescence assay (IFA) results, were analyzed by both BioCLIA® and ELISA (from an internationally renowned manufacture). Sensitivity, specificity and total agreement of the compared assays were analyzed. Lastly, a total of 240 clinically characterized samples were used to study clinical sensitivity and specificity, including 40 patients for WG, 50 patients for Systemic Lupus Erythematosus (SLE), 50 patients for Rheumatoid Arthritis (RA), and 100 samples for healthy donors from local Chinese hospitals. **Results:** The BioCLIA® anti-PR3 assay performed good linearity ranging from 2-400 RU/mL and the LOD was 0.17 RU/mL. In the precision study, the CV% was 3.98% for intra-assay and 5.24% for inter-assay, respectively. Bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), lipid (up to 2000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL) did not affect the detection of anti-PR3 IgG in serum. In a clinical evaluation, using 100 clinical samples with IFA assay confirmed results, we found the BioCLIA® has higher sensitivity of 95.0% (57/60) than ELISA result of 91.7% (55/60), but with a similar specificity of 98.5%. The total agreement of BioCLIA® & ELISA compared to IFA assay were 96.0% (96/100) & 94.0% (94/100), respectively. From the clinical study in Chinese patients, the positive rate showed on BioCLIA® anti-PR3 assay in WG, SLE, RA and healthy donors were 95.0% (38/40), 6.0% (3/50), 1.6% (1/50) and 1.0% (1/100), respectively. **Conclusion:** BioCLIA® anti-PR3 assay is an innovative semi-quantitative assay which exhibits fast and accurate analysis and demonstrates linearity in an extended analytical measuring range as well as good reproducibility. In the aspect of clinical comparison, the kit offered a better clinical relevance when compared with ELISA, and a good agreement with IFA assay, which is considered as the gold standard method. It serves as a promising and fully automated alternative for IFA assay in the detection of anti-PR3 IgG antibodies and valuable to aid in the diagnosis of WG.

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Analytical and Clinical Performance of the BioCLIA® Procalcitonin Assay

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Background: Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin. PCT helps differentiate bacterial from viral infections, the early detection of an elevated PCT level in patients with suspected bacterial infections enabling earlier antibiotic treatment, so PCT has been widely used as a biomarker of bacterial infection or sepsis. PCT also supports informed decisions on whether continue, change or stop antibiotics, improving patient care and decreasing antibiotic misuse and resistance. The BioCLIA® PCT kit is designed for the specific, quantitative detection of PCT in serum. HOB BioCLIA® chemiluminescent immunoassay analyzer is a random-access, high-throughput, continuous automated measurement platform. **Methods:** In our study, the analytical performances of BioCLIA® PCT assay including the limit of detection (LoD), limit of quantitation (LoQ), intra-assay, inter-assay and interference studies were evaluated according to CLSI guidelines. Total of 476 clinical samples collected from major Chinese hospitals were analyzed and compared with the same kit on Roche Cobas e 601 system. **Results:** The LoD of BioCLIA® PCT was 0.0032 ng/mL, while the LoQ was 0.02 ng/mL. The linear range of the PCT assay is 0.02-100 ng/mL. Precision studies demonstrated acceptable CV% of 4.2% & 8.8% for intra-assay and inter-assay for PCT assay. Total of 476 clinical samples were compared between Roche Cobas e 601 and BioCLIA® assays. The regression analysis for PCT with Passing-Bablok regression fit of BioCLIA®=1.044 Cobas e 601-0.09 (r=0.985). Bilirubin (up to 25 mg/dL), hemoglobin (up to 900 mg/dL), lipid (up to 1500 mg/dL), human katacalcin (up to 30 ng/mL), human calcitonin (up to 10 ng/mL), human alpha-CGRP (up to 10000 ng/mL), human beta-CGRP (up to 10000 ng/mL), HAMA (up to 2000 ng/mL), and RF (up to 1000 IU/mL) did not affect the PCT qualitative detection in serum. **Conclusion:** The BioCLIA® PCT assay performs an extended working range and good precision & reproducibility. Excellent correlation of results is observed between BioCLIA® PCT and Roche Cobas e 601 systems. It provides the fast & accurate detection of PCT in serum and can be used as an aid in the detection of bacterial infections and sepsis.

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Presence of anti-cN-1A (Mup44, NT5c1A) IgG is specific for Sporadic Inclusion Body Myositis

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Background: Sporadic Inclusion Body Myositis (sIBM) is an autoimmune disease manifesting with muscle degeneration, inflammatory infiltrates and inclusion vacuoles. Diagnosis of sIBM is hampered by its imprecise characteristics, at times indistinguishable from other Idiopathic Inflammatory Myopathies, but may now be assisted by detection of sIBM-specific autoantibodies targeting muscle antigen Mup44, identified as cytosolic 5'-nucleotidase 1A (cN-1A; Mup44; NT5c1A). This study evaluated sensitivity and specificity of an anti-cN-1A IgG serological assay in sera from patients with and without sIBM. **Methods**: Serum from patients with clinically and pathologically diagnosed sIBM (n=68), suspected sIBM (n=15), myositis controls [including dermatomyositis (n=4), polymyositis (n=7); unspecified myositis without sIBM (n=94), muscle atrophy (n=1), myonecrosis (n=4)], from patients with SLE (n=33), scleroderma (n=20), Sjogren's (n=20), rheumatoid arthritis (n=20) and from healthy controls (n=254) were tested for anti-cN-1A IgG using an anti-cN-1A ELISA (full-length antigen, Euroimmun AG). **Results:** Anti-cN-1A was most frequent among definite sIBM (41.2%). The overall specificity was 96.3% with individual specificities from 90% (scleroderma) to 100% (PM or DM). **Conclusion:** The presence of anti-cN-1A in serum appears to be disease-specific for sIBM. These antibodies are found at a moderate prevalence, but are only rarely detected in other autoimmune conditions. Thus, anti-cN-1A ELISA may support the diagnostics of sIBM and accelerates the suspected diagnosis in cases of positivity, where muscle biopsy is delayed or unfeasible.

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Comparison of two multiple allergen simultaneous tests: AdvanSure Alloscreen and PROTIA Allergy-Q

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Background: Multiple allergen simultaneous test (MAST) has been widely performed as a screening tool for detecting allergen specific immunoglobulin (Ig) E. However, since MAST has no standard reference method, comparisons with existing assays would be practical to assess the performance of the new one. Recently, PROTIA Allergy-Q (ProteomeTech, Seoul, Korea), a new automated analyzer with high-throughput for MAST, has been introduced in South Korea. In this study, we compared the performance of PROTIA Allergy-Q with MAST assay currently utilized to evaluate its usefulness in clinical laboratories. **Methods:** Sixty serum samples with positive results in AdvanSure Alloscreen (LG Life Sciences, Seoul, Korea) (30 for food and 30 for inhalant panel) were subjected to PROTIA Allergy-Q. We assessed positivity rates and percent agreements according to allergen panel or each allergen. 267 ImmunoCAP tests (Phadia, Uppsala, Sweden) were repeated with sera demonstrating discrepancies between two MAST assays. **Results:** The positivity rates of inhalant panel and food panel were 35.8% and 31.2% by PROTIA Allergy-Q, 36.8% and 33.5% by AdvanSure Alloscreen, respectively. Percent agreements were 83.3 % (κ, 0.661) and 84.9% (κ, 0.671) between PROTIA Allergy-Q and AdvanSure Alloscreen. However, 19 allergens in inhalant panel and 13 allergens in food panel showed concordant rates below 80% ranging from 46.7% to 76.7%. The agreement of PROTIA Allergy-Q and ImmunoCAP (69.3%) results was superior to one of AdvanSure Alloscreen with ImmunoCAP (47.9%) results. **Conclusion:** Our study demonstrated that PROTIA Allergy-Q showed a good agreement with current MAST assay and better agreement with ImmunoCAP assay than current MAST assay.

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Lowest is not always the best: An international serum protein electrophoresis accuracy study

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Background: Serum protein electrophoresis (SPEP) is the standard for the diagnosis and therapeutic monitoring of patients with monoclonal gammopathies. Significant imprecision and inaccuracy are documented when M-proteins are <1g/dL, posing challenges in reporting small M-proteins. Using therapeutic monoclonal antibody-spiked serum, we aim to assess SPEP accuracy across four laboratories in four countries. **Methods:** Serum with normal (0.6-1.5g/dL), hypo- (<0.5g/dL) or hyper-gamma (>1.7g/dL) backgrounds were spiked with daratumumab, Dara (cathodal migrating), or elotuzumab, Elo (central-gamma migrating), with concentrations from 1.0-0.0125g/dL (n=62). Provided with total protein (reverse biuret, Siemens), laboratories blindly analyzed samples according to their SPEP standard operating procedures. Three laboratories used agarose gel electrophoresis (AGE) (one Helena, two Sebia) and one used capillary electrophoresis (CZE) (Sebia). All laboratories used perpendicular drop gating for quantitation. An all method mean percent recovery range of 80-120% was set as acceptable. Inter-assay imprecision CV was calculated in one institution with 10 replicates. **Results:** An all method mean percent recovery was determined for each sample set (n=16), with unacceptable recoveries bolded in Table. Imprecision was assessed using AGE-Helena. Quantifying at 1g/dL, the mean imprecision was 1.9% (range 1.2-2.2%) for all pools while at 0.2g/dL, imprecision was 6.4% (2.7-12.3%). **Conclusions:** Gammaglobulin-background, migration location and concentration all affect the imprecision and accuracy of quantifying M-proteins by SPEP. As the background increases, imprecision increases and accuracy decreases leading to significant overestimation of M-protein quantitation especially evident in hypergamma-samples. Cathodal-migrating M-proteins were associated with less imprecision and higher accuracy compared to central-gamma migrating M-proteins, which is attributed to the increased gamma-background contribution in M-proteins migrating in the middle of the gamma fraction. Additionally, there is a greater rate of imprecision and loss of accuracy at very low M-protein concentrations. This study suggests that quantifying exceedingly low concentrations of M-proteins, although possible, may not be the optimal practice for adequate precision and accuracy.

Table: All method mean recovery for each sample set.

	Mean Recovery, % (Range) (N)					
Spiked M-protein (g/dL)	Dara-Hypo	Dara-Normal	Dara-Hyper	Elo-Hypo	Elo-Normal	Elo-Hyper
1.0	87 (79-91) (16)	92 (77-100) (16)	110 (89-122) (16)	81 (54-98) (16)	96 (62-116) (16)	**125 (84-149) (16)**
0.8	88 (73-96) (16)	93 (71-104) (16)	122 (88-141) (15)	84 (54-100) (16)	97 (60-116) (16)	**138 (91-167) (16)**
0.6	87 (61-97) (16)	92 (70-107) (16)	128 (83-148) (15)	90 (55-110) (16)	106 (63-129) (16)	**158 (108-187) (16)**
0.5	88 (65-99) (16)	100 (74-113) (16)	**138 (89-161) (15)**	86 (56-102) (16)	110 (62-136) (16)	**162 (102-214) (16)**
0.4	89 (58-110) (16)	104 (55-132) (16)	**154 (95-179) (15)**	92 (61-111) (16)	118 (71-151) (16)	**187 (124-238) (16)**
0.3	89 (50-113) (16)	109 (73-131) (16)	**169 (105-214) (15)**	93 (53-116) (16)	**136 (88-163) (16)**	**203 (135-269) (16)**
0.2	98 (70-117) (16)	**124 (65-156) (16)**	**225 (127-317) (15)**	105 (67-128) (16)	**156 (93-197) (16)**	**245 (155-368) (16)**
0.1	**127 (109-146) (4)**	**160 (100-229) (5)**	**218 (185-251) (3)**	**139 (121-157) (5)**	**207 (110-279) (16)**	**273 (175-374) (6)**
0.05	**153 (122-186) (3)**	**223 (201-245) (2)**	**260 (260-260) (1)**	**184 (173-194) (5)**	**314 (280-347) (2)**	**482 (200-764) (3)**
0.025	**198 (187-208) (2)**	**0 (0-0) (0)**	-	**0 (0-0) (0)**	**407 (308-508) (2)**	-
0.0125	**0 (0-0) (0)**	**0 (0-0) (0)**	-	**0 (0-0) (0)**	**0 (0-0) (0)**	-

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Study of the frequency of test positivity for Arboviruses in a 5-year period in the city of São Paulo

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Background: Arboviruses represent a growing problem for the propagation potential characterized by easy adaptability in hosts. The chances of epidemics are extensive due to universal susceptibility and their numbers are worrisome due to the presence of large numbers of severe and fatal cases, or with neurological, articular and hemorrhagic involvement. In the Brazilian epidemiological context, the highest circulating arboviruses are dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV), although there are others with potential for dissemination in the country.This framework requires coping with broad-spectrum policies and interventions involving all public health, especially the need for efficient and rapid laboratory tests that diagnose and monitor patients. In this study the authors aimed to measure the number of positive cases by the available tests and the number of requests for arboviruses: DENV, CHIKV and ZIKV, in the last 5 years in São Paulo. **Methods** - Results of requests for DENV, CHKV and ZIKV tests, of 5 years (2013 to 2017), were analyzed in the database of a large laboratory in the city of São Paulo. The study was retrospective and observational. The tests were performed using the following methods: DENV-(ELISA)-Panio Focus Novagnost, Euroimmun / Siemens®, Immunochromatography(WAMA),CHKV(ELISA)-Euroimmun) and ZIKV- Immunochromography - OrangeLife and PCR In real time. **Results:** From the total of 59793 arboviruses test requests for the studied period, 26617 positive cases were shown represented in the several tests in Table 1. **Conclusion**- Based on the data found, that as the years passed, the types of arboviruses increased. Until 2015, there was practically no request for tests for CHKV or for ZIKV. From 2016 these last arboviruses began to present positivity. We conclude that arboviruses are emerging in Brazil and that several deseases, such as Yellow Fever, may appear.

Table 1: Representation of the positive cases for arboviruses in the different tests used in laboratory routine.

	Positivity % per year					
	2013 N/%	2014 N/%	2015 N%	2016 N/%	2017 N/%	Total N/%
DENV- IgM	0 0	1 0.04	1121 51.46	680 31.22	376 17.26	2178
DENV- IgG	0 0	0 0	98 15.19	305 47.28	242 37.51	645
DENV-fast test	2283 9,97	5709 24.93	8802 38.45	4916 21.47	991 4.32	22891
ZIKV - IgM	0 0	0 0	0 0	0 0	216 100	216
ZIKV - IgG	0 0	0 0	0 0	22 29.77	52 70.27	74
ZIKV - PCR	0 0	0 0	0 0	34 73.91	12 26.08	46
CHKV - PCR	0 0	0 0	0 0	5 62.50	3 37.50	8
Antigen- DENV NS1	0 0	0 0	0 0	0 0	559 100	559

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Optimization of resources in the clinical laboratoy: Impact in the safety of the patients

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Introduction: The Clinical Laboratory is in permanent evolution and should not be limited to providing results as a data factory. The results should be interpreted in the clinical context of each patient. In addition, laboratory techniques and protocols should be applied to help the clinician to diagnose the patients in the shortest time. The introduction of comments that guide the clinicians and the use of additional tests in patients with unexpected analytical findings will allow the clinicians to take the correct diagnostic decisions. **Case presentation:** A 63-year-old woman was admitted to the emergency services due to heavy bleeding after tooth extraction. She reported recurrent hemorrhages since the dental intervention together with fatigue and dyspnea. Furthermore, the patient presented back bone pain. The following parameters were altered in the laboratory report: Hemoglobin=8.7 g/dL, calcium=11.15 mg/dL and total serum proteins=13.70 g/dL. The patient was referred to her primary physician to study the source of the anemia. A hyperproteinemia (13.2 g/dL) was detected in the Laboratory accompanied with anemia (7.8 g/dL) and hypercalcemia (11.15 mg/ dL). Furthermore, a marked Rouleaux phenomenon is observed in the peripheral blood smear. With these clinical findings associated with multiple myeloma (MM), the protocol established for the screening of a monoclonal gammopathy (MG) is applied. In the serum protein electrophoresis was observed a well-defined monoclonal peak of 6.9 g/dL. The immunoglobulin levels were IgA=6856 mg/dL, IgG=198 mg/dL and IgM=6 mg/dL. The large increase in IgA levels was associated to a marked decrease in IgG and IgM levels. According to our protocol, serum free light chains (FLC) were quantified. A K/L ratio of 0.03 was obtained showing lambda monoclonality (Kappa=4.93 g/dL, Lambda=170.26 mg/L). Following up the protocol, a serum immunofixation was performed identifying the monoclonal component as IgA-Lambda. With these findings, the clinical case was presented in the Unit of Monoclonal Gammopathies (UGAM) of the Hospital to inform to the clinicians. The UGAM is a multidisciplinary group formed by specialists from different clinical specialties involved in the study of patients with MG (Clinical Biochemistry, Hematology, Nephrology, Pathology, Cardiology, Osteoporosis, Neurology and Rheumatology). The patient is referred to the Hematology Unit to complete the study. A bone marrow study showed a 10% of plasma cells with pathological phenotype (98.9% with lambda clonality) and several bone lesions were observed in the PET-CT scan. With these clinical data the patient was diagnosed of IgA-lambda MM Stage II by the hematologist. **Conclusions:** The laboratory has a essential role in the diagnosis of patients with MG. In the context of clinical symptoms associated to MG (bone pain, pathological fractures, anemia, and hiperproteinemia), the application of this protocol allowed us to identify the presence of a monoclonal component in the patient. The quickly communication of the results to the clinicians at the UGAM meeting helped a rapid diagnosis of the patient. Communication between professionals from different clinical units is essential in daily practice for a correct interpretation and proper use of laboratory findings. Patient safety must be a priority in the Clinical Laboratory.

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Performance Evaluation of the Newly Developed HOB BioLine® Pro Automated Allergen-Screen Assay

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Background: A new multiple allergen simultaneous test on the immunoblot has recently been developed that is simple, rapid, and economical, and requires a small amount of serum samples. The BioLine® Pro Allergy Screen assay is an advanced multiplex test that allows for simultaneous detection of specific IgE (sIgE) against multiple allergens on the fully automated instrument including the sample addition and data interpretation. In this newly developed assay, specific IgE from the patient sample is captured by the allergen coated to the nitrocellulose strip. After washing, the biotinylated anti-IgE is added and incubated in the trap. Excess biotinylated anti-IgE is removed by the next wash step and then streptavidin labeled with alkaline phosphatase is added. It forms the complexes consisting of allergen/ specific IgE/ biotinylated anti-IgE streptavidin conjugate. The purple color is developed on the strip by the addition of substrate solution. The test results are recorded after air drying and image scanned by an integrated camera automatically. We evaluated the specific antigen detection results from multiple allergen simultaneous tests between ImmunoCAP and BioLine® Pro systems. **Methods:** In this study, the analytical performance of Allergen-specific IgE to 10 inhalant allergens including D1 (*Dermatophagoides pteronyssinus*), D2 (*Dermatophagoides farina*), E1 (Cat epithelium), E5 (Dog dander), H1 (House dust), M6 (*Alternaria alternata*), T3 (Common silver birch), W1 (Common ragweed), W21 (Wall pellitory), W6 (Mugwort) and 10 food allergens including F14 (Soybean), F20 (Almond), F202 (Cashew nut), F27 (Beef), F3 (Codfish), F31 (Carrot), F5 (Rye), F84 (Kiwi), F85 (Celery), F95 (Peach) was evaluated including precision and sensitivity according to the CLSI guideline. Total of 136 clinical samples was evaluated by both of HOB BioLine®Pro Allergy Screen assay and ImmunoCAP systems. **Results:** The CV%s of D1, D2, E1, E5, H1, M6, T3, W1, W21, W6, F14, F20, F202, F27, F3, F31, F5, F84, F85 and F95 were in a range from 4.47~7.87% for within-run, and 5.58~8.33% for total-run. Bilirubin (up to 20 mg/dL), hemoglobin (up to 40 mg/dL), and lipid (up to 2000 mg/dL) did not affect the IgE qualitative detection in serum.

It showed no cross-reactivity with other human classes of immunoglobulins (IgG, IgD, IgA and IgM) at physiological concentrations. Total of 136 clinical samples were compared between ImmunoCAP and BioLine®Pro Allergen screen assay at the cut-off of 0.35 kU/L. The total sensitivity, specificity and concordance for the twenty allergens was 86.57% (928/1072), 92.42% (1523/1648) and 90.11% (2451/2720), respectively. **Conclusion:** With a good precision and excellent agreements with ImmunoCAP, HOB BioLine® Pro Allergy screen assay provides the fast & accurate IgE detection *in* patients. It runs 50 strips simultaneously and can get 1000 results within 88 minutes. It can be an efficient way of testing for specific allergens in the clinical laboratory or at the physician's office and hospitals with large volume of samples.

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Measurement of C1 inhibitor protein using the Optilite® automated turbidimetric analyser

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C1-INH (C1 inactivator / C1 inhibitor) is a protease inhibitor which controls activation of the classical complement pathway, fibrinolytic, clotting, and kinin pathways. The Optilite® C1 inactivator kit is intended for the quantitative in vitro measurement of C1-INH in serum, lithium heparin and EDTA plasma using the Binding Site Optilite analyser. C1-INH deficiency causes an increase in bradykinin, leading to vasodilation, fluid extravasation and ultimately angioedema, and can be hereditary (HAE) or acquired (AAE). It is important to distinguish between angioedema related to C1-INH and that caused by other mechanisms, as treatment options are different. In both HAE and AAE, C1-INH protein concentration is used alongside C1-INH functional tests and C4 concentration to aid in patient diagnosis. Here we describe a fully automated assay for the measurement of C1-INH protein concentration on the Optilite turbidimetric analyser. A linearity study was performed based on CLSI guideline EP6-A. The linearity of this assay was confirmed over 0.07 - 0.47 g/L at the standard 1+4 analyser dilution. A precision study based on CLSI guideline EP05-A2 was performed over 21 days. 5 samples with different C1-INH concentrations (0.12-0.41 g/L) were run in duplicate, with two runs per day using 3 reagent lots and 5 different analysers. The within run, between run, between day, between batch and between instrument percentage coefficients of variation (%CVs) were all <6%. The total %CV was <8% in all 5 samples. A 95th percentile reference interval of 0.21-0.38 g/L was generated by measuring C1-INH in serum samples from 120 healthy adult blood donors (median 57 years; range 23-94). A comparison of C1-INH concentration was made between the Optilite assay and a clinically used predicate assay using 260 clinical samples. There was a strong correlation between the assays (R^2=0.94, p<0.0001, Passing and Bablok slope y=0.83x), with 99.6% agreement in determining whether samples were above or below the lower limit of the normal range. In conclusion, the Optilite C1 inactivator assay allows the automated and precise quantification of C1-INH concentrations in patient sera samples and it correlates well with existing methods. It could therefore be used as a tool to aid in the investigation of the cause of angioedema.

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Anti-parietal cell antibodies a frontline marker in Primary Care

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Background: There is a high prevalence of vitamin B12 deficiency. Anti-parietal cell antibodies (APCA) and/or intrinsic factor blocking antibodies (IFBA) can diagnose autoimmune gastritis atrophy (AAG) which increases the risk of gastric carcinoma. The aim is to study the frequency of APCA and IFBA in primary care patients with severe vitamin B12 deficit, and to correlate with demographic characteristics and hematological values. **Methods:** An observational study was designed and conducted from 1st May to 30th September 2017 in a community University Hospital covering a Health Department (HD). Participants were Primary care patients of the HD. In consensus between the laboratory and General Practitioners (GPs) an intervention was designed that consisted in the laboratory information system automatically registering APCA and IFBA to the laboratory request of any primary care patient with a new s-vitamin B12 < 73.8 pmol/L (severe vitamin B12 deficit). We studied the number of patients with APCA and/or IFBA positivity, and their demographic data, MCV and haemoglobin values. APCA and IFBA were performed using the EliA immunoassay on the Phadia 2500 system according to the manufacturer's instructions (Phadia GmbH, Freiburg, Germany). **Results:** There were 77 new cases of severe vitamin B12 deficit. Among those, 44 (57.1%) were APCA+, and 11 (14.3%) were IFBA+. Age and sex did not significantly differ among patients with positive or negative antibodies. Table shows percentage of patients showing anemia or macrocytosis regarding the results of APCA and IFBA. **Conclusion:** The automated strategy to identify subjects with APCA+ and or IFBA+ seemed successful given they efficiently detected APCA positivity in more than half of patients with severe vitamin B12 deficiency. The positivity would diagnose AAG and indirectly would confirm vitamin B12 deficit without confirmatory tests, turning APCA into a frontline marker in primary care.

		ANEMIA		MCV	
		NO	YES	<100fL	>100fL
APCA	Negative	19 (36.5%)	14 (56.0%)	21 (40.4%)	12 (48%)
	Positive	33 (63.5%)	11 (44.0%)	31 (59.6%)	13 (52%)
IFBA	Negative	43 (82.7%)	23 (92.0%)	46 (88.5%)	20 (80.0%)
	Positive	9 (17.3%)	2 (8.0%)	6 (11.5%)	5 (20.0%)

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Performance characteristics of the VaccZyme Salmonella Typhi Vi IgG commercial ELISA

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Background: Antigen-specific serum IgG measurements are used to assess immune system competence and recovery, and may be used to support treatment decisions. Evaluation of adaptive immunity requires initiating B cell stimulation with both protein and polysaccharide vaccines. The current gold standard polysaccharide vaccine used for assessment is Pneumovax®23 but interpretation can be complicated. Typhim Vi® is a Vi capsular polysaccharide vaccine administered to populations at risk of typhoid fever, *i.e.* in areas of endemic typhoid fever or to individuals travelling to such areas. Recent reports suggest that measurement of the IgG response to Typhim Vi may have utility in supporting a diagnosis of antibody deficiency. Typhim Vi has now been included in the recommendations for use as a diagnostic vaccine. Here we describe the performance of the VaccZyme™ human anti-Salmonella typhi Vi IgG ELISA which has been developed for the measurement of typhi Vi IgG. The performance characteristics from the production of six consecutive different kit batches are reported. **Methods:** The concentration of Typhi Vi IgG was measured using the VaccZyme human anti-Salmonella typhi Vi IgG ELISA (The Binding Site Group Ltd., Birmingham, UK) in serum samples obtained from 40 adult blood donors (15 male and 25 female, aged 18-66 years). The measuring range of the assay was 7.4-600U/mL. **Results:** Variation in coating of the microtitre plates with Typhim Vi® was assessed. Median coefficient of variation (CV) for coating each individual batch was <5%. Median CV from six independent batches was 4% (n=24; range 3.3-5%). Target concentration recoveries of 10 samples were assessed for each batch. The percentage recoveries for typhi Vi IgG concentrations ranged from 96-105%. Typhi Vi IgG concentration comparisons between the different batches were performed using 30 serum samples. A median Passing Bablok regression of 1.05 (range 0.97-1.11) was obtained with a median linear regression correlation coefficient of r=0.99 (range 0.97-0.99). A precision study was performed for each of the six batches using 10 samples with analyte concentrations between 11.5-433 U/mL. The between run coefficients of variation (CVs) were 5-10% for all samples. **Conclusion:** The VaccZyme anti-Salmonella typhi Vi ELISA provides a reliable and precise method for quantifying Typhi Vi IgG in human serum with high batch-to-batch reproducibility.

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Evaluation of Two Fecal Calprotectin Assays

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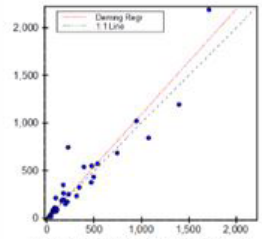
Background: Fecal calprotectin is a valuable noninvasive marker for diagnosis and management of inflammatory bowel disease (IBD). The aim of our study is to evaluate the performance of two commercially-available and FDA-approved fecal calprotectin assays. **Method:** The Quanta Lite Calprotectin ELISA and Calprotectin Extended Range assay were obtained from Inova Diagnostics (San Diego, CA). Both assays were performed per manufacturer's instructions. Analytical performance was evaluat-

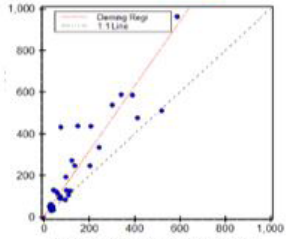
ed following Clinical and Laboratory Standards Institute guidelines. Residual patient stool samples for calprotectin tests were used in the evaluation. Discordant results were assessed based on medical record review. **Results:** As shown in the table, the Calprotectin ELISA has better precision than the Extended Range assay. However, the Extended range assay showed much wider analytical measurement range (AMR), eliminating the need for additional dilution of samples. The measured calprotectin levels were categorized into normal (<50µg/g) and abnormal (>50µg/g). The agreement with a reference lab method using the PhiCal assay was 97.6% and 91.0% for Calprotectin ELISA and Extended Range assay respectively, and 94.1% between the Calprotectin ELISA and Extended Range assay. Although both methods correlated well with the reference lab method in quantitation, the slope and intercept of the Extended Range assay differed extensively from the other two assays. A large positive bias (about 50%) was observed between the calprotectin levels generated from the Extended Range and the reference lab method. **Conclusion:** Both assays showed acceptable agreement in identification of normal verses abnormal. However, calprotectin results generated from different assays are not comparable because of the large quantitative differences.

	Quanta Lite ELISA		Extended Range Assay	
Precision	Conc.	Total CV	Concentration	Total CV
Control-L	72.8	2.8	280.6	3.6
Control-H	141.3	2.7	1267.2	5.8
Patient	326.8	9.0	279.2	14.6
AMR	15.6-220 µg/g		27.1-3000 µg/g	

Method comparison with a reference lab method using PhiCal assay



X: Reference lab method;
Y: Calprotectin ELISA



X: Reference lab method;
Y: Extended Range Assay

R	0.9485	0.8992
Bias (%)	21.1 (6.88%)	95.3 (46.1%)
Slope (95% conf)	1.1 (1.0 to1.2)	1.6 (1.3 to 1.8)
Intercept (95%conf)	-10.0 (-68.8 to 48.8)	7.1 (-51.1 to 65.2)

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Impact of obstructive sleep apnea on innate immune response and viral RNA quantitation in influenza virus infection: from host gene expression to primary cellular model

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Background: Influenza virus infections can lead to acute respiratory distress syndrome and death in humans. Old age, male sex, medical comorbidities, and obesity are risk factors for the development of severe disease. These characteristics are also associated with obstructive sleep apnea (OSA). OSA may further compromise host immune responses. However, the impact of OSA in influenza infection has not been reported. This study included two parts: 1) comparison of host gene expression of the upper respiratory tract (URT), with special regard to immune response, in patients with different severity of OSA, and 2) assessment of the effects of OSA on cytokine levels and influenza RNA quantity in primary cultures of human URT epithelial cells after H7N9 infection. **Methods:** *Part 1:* Genomic RNA extracted from the fresh tissue of the uvula from 10 middle-aged patients without evident virus infection, 5 severe OSA (apnea-hypopnea index [AHI] ≥ 30) patients and 5 sex- and body weight-matched patients with mild OSA (AHI < 15), was used in RNA-Seq analysis. Differentially expressed genes (DEGs) were analyzed and only the genes with false discovery rate ≤ 0.001 and the absolute value of Log_2Ratio ≥ 1 were considered for annotation analysis of gene ontology. Pathway analysis was performed in MetaCore™ to derive functional annotations. *Part 2:* Primary epithelial cells of the URT harvested from another 17 middle-aged patients undergoing airway surgery (10 severe OSA patients and 7 controls without evident OSA) were experimentally infected with H7N9 for 72 h. After virus infection, the culture media were collected for cytokine detection using the Bio-Plex Pro™ Human Cytokine 27-plex panel and viral RNA quantitation using real-time reverse transcription polymerase chain reaction at 24 h and 72 h. **Results:** *Part 1:* There were 13 down-regulated and 45 up-regulated DEGs between severe OSA group and mild OSA group. Of them, 18 DEGs involved in immune system process. Furthermore, 86 significantly enriched pathways of immune response category including stress-induced antiviral cell response (ratio 54/57, P = 1.3e-13) and innate immune response to RNA viral infection (ratio 23/28, P = 4.0e-4) were identified. *Part 2:* At 24 h post infection, levels of IL-4, IL-6, IL-10, IFN-γ, MCP-1, and VEGF in patients with severe OSA were significantly higher than those of controls (all P < 0.05). The impact of OSA on cytokine levels was not evident at 72 h. Furthermore, the viral RNA quantities in primary epithelial cells from severe OSA patients and controls did not differ significantly at 24 h and 72 h, respectively. **Conclusion:** Our preliminary findings demonstrate for the first time a clear distinction of host gene expression in regard to antiviral cell response and innate immune response to RNA viral infection between severe OSA patients and mild OSA patients. Although H7N9 infection of primary epithelial cells harvested from severe OSA patients can trigger rapid increases of several cytokines; however, these immune responses do not suppress viral replication. Our results suggest that OSA can potentially impact on innate immune response in influenza virus infections.

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Bronchoalveolar lavage findings in the diagnosis of patients with interstitial lung diseases

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Background: Interstitial lung diseases (ILD) are a group of diseases were the main pathological changes affecting the alveolar structures. The diagnosis of the patients are based on clinical symptoms, pulmonary function tests and radiological studies. When the diagnosis in unclear, invasive tests like bronchoalveolar lavage and pulmonary biopsy are used. The study of the bronchoalveolar lavage fluid in some interstitial lung diseases can reveal typical patterns to each disease that can support the diagnosis. The objective of this study was to perform a descriptive analysis of the cytologic study (lymphocytes, neutrophils, histiocytes and eosinophils) and the lymphocyte subpopulations in bronchoalveolar lavage fluid from patients with interstitial lung disease. **Methods:** Retrospective study of the bronchoalveolar lavage fluids of 58 patients with ILD: sarcoidosis (SAR) (n=10), idiopathic pulmonary fibrosis (IPF) (n=12), non-specific interstitial pneumonia (NSIN) (n=20), cryptogenic organizing pneumonia (COP) (n=7), and extrinsic allergic alveolitis (EAA) (n=9). The bronchoalveolar lavage fluid was analyzed to determine the distribution of cell populations and the lymphocyte subsets: $CD3^+$, $CD19^+$, $CD4^+$, $CD8^+$, $CD3^+CD4^-CD8^-$, and $CD3^-CD16\&56^+$.The cell populations and the lymphocyte subsets were determined in a FACS Canto II Flow Cytometer. Values of cell populations and lymphocytes subsets were given in percentages (%). **Results:** The distribution of cell populations in bronchoalveolar lavage classified the interstitial lung diseases in three groups. Isolated lymphocytic alveolitis was found in SAR and isolated neutrophilic alveolitis was found in COP and IPF. Mixed alveolitis was the most common pattern in EAA and NSIN. The CD4:CD8 ratio was the most useful parameter in our study. The ratio was high in SAR (median, 5.80) and it was inverted in EAA (median, 0.19). It was low in the other interstitial lung diseases, with median values of 1.03 in IPF, 1.00 in NSIN and 1.07 in NOC. NK cells populations were higher in NOC (median, 28.00) than the others diseases with median values of 3.00 in SAR, 2.00 in EAA, 4.50 in IPF and 3.00 in NSIN. **Conclusions:** The study of the bronchoalveolar lavage fluid parameters in association with clinical and radiologic data help us to discriminate between interstitial lung diseases. The CD4:CD8 ratio can discriminate sarcoidosis from the other interstitial lung diseases. NK cell populations can discriminate NOC from the others interstitial lung diseases. The bronchoalveolar lavage fluid should be considered a very useful tool in the diagnosis of the patient.

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Evaluation of a Novel Multi-Analyte Assay for the Detection of Autoantibodies in the Diagnosis of Antiphopholipid Syndrome (APS)

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Background: Antibodies to antiphospholids (aPL) and associated proteins are a hallmark in the diagnosis of antiphospholipid syndrome (APS). Recently, a novel fully automated paramagnetic particle based multianalyte system (PMAS) has been developed which allows for the detection of autoantibodies to cardiolipin (aCL), beta 2 glycoprotein I (aβ2GPI), and to the phosphatidylserine/prothrombin (aPS/PT) complex. This study aimed to analyze the clinical performance of the novel system and compare to reference methods using clinically characterized samples.**Methods:** A total of 279 samples were collected from APS patients and various other disease controls (n=228) and were tested for aCL and aβ2GPI. Out of these samples, 104 from APS patients and 36 from disease controls were tested for aPS/PT. Antigens were coupled to paramagnetic particles and tested using PMAS (research use only, Inova Diagnostics, San Diego, CA). A reduced number of samples in the patient population were also tested by reference methods for comparison studies (QUANTA Flash CIA and QUANTA Lite ELISAs, Inova Diagnostics, San Diego, CA). Clinical sensitivity and specificity was calculated for all methods and comparative analysis was performed on the predicate device. **Results:** The clinical performance for the novel aCL and aβ2GPI PMAS assays are outlined in the table below. Interestingly, for the first time we show good discrimination between APS and controls using the aPS/PT IgA isotype. Among the APS patients tested for all markers (aCL, aβ2GPI, aPS/PT), 50.0% of patients were aPS/PT positive only (negative for aCL and aβ2GP1). The correlation between platforms was good for aCL, aβ2GP1, and aPS/PT assays for all isotypes. The total agreement between aPS/PT assays is outlined in the table below.

Characteristic	aCL IgG	aβ2GPI IgG	aCL IgA	aβ2GPI IgA	aCL IgM	aβ2GPI IgM
Sensitivity (95% CI)	67.0% (61.3-2.3%)	41.6% (35.9-7.4%)	43.4% (37.7-49.2%)	38.4% (32.8-44.2%)	22.9% (18.4-28.2%)	21.5% (17.1-26.7%)
Specificity (95% CI)	90.8% (96.3-93.9%)	97.4% (94.4-8.8%)	98.2% (95.6-99.3%)	97.4% (94.4-98.8%)	97.8% (95.0-99.2%)	98.2% (95.6-99.3%)
LR+	7.3	15.8	24.7	14.6	10.5	12.3
LR-	0.36	0.60	0.58	0.63	0.79	0.80
Odds ratio (95% CI)	20.0 (12.0-33.4)	26.3 (11.5-60.0)	42.9 (16.1-134.1)	23.0 (10.1-52.5)	13.3 (5.4-32.7)	15.3 (5.7-41.3)
Youden's index	0.58	0.39	0.42	0.36	0.21	0.98

	PMAS PS/PT IgG vs. QL PS/PT ELISA	PMAS PS/PT IgM vs. QL PS/PT ELISA IgM	PMAS PS/PT IgA vs. QL PS/PT ELISA IgA
Total Percent Agreement (95% CI)	80.0% (72.6-95.0%)	79.3% (71.8-85.2%)	89.4% (83.2-93.4%)
Kappa (95% CI)	0.58 (0.43-0.72)	0.57 (0.43-0.70)	0.65 (0.49-0.81)

Conclusion: Our data show excellent analytical and clinical performance of the PMAS as an aid in the diagnosis of APS. The addition of aPS/PT in the new system holds promise in the improvement for diagnosis and patient stratification.

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Immunogenicity of anti-TNF therapy in inflammatory bowel diseases

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Background: The treatment of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) has improved substantially with the introduction of biological therapy based on inhibitors of tumor necrosis factor alpha (anti-TNF): Infliximab (IFX) and Adalimumab (ADA). However, the presence of anti-drug antibodies (anti-Infliximab and anti-Adalimumab) is associated with clinical relapse of the disease and increased morbidity. A percentage of these patients on treatment with anti-TNF therapy have an active disease either because the treatment does not initiate a response (primary failure) or they have an initial response but there is a lack of response over time (secondary failure) due mainly to to the development of anti-drug antibodies. The objectives of the study are: i) to evaluate the presence of anti-drug antibodies in patients with IBD, ii) to evaluate the relationship between the presence of anti-drug antibodies and the clinical response, iii) to determine the circulating levels of drug in patients without antibodies according to the degree of activity of the disease. **Materials and methods:** Observational descriptive study performed during a period of 9 months in a group of 86 patients with IBD: 58 patients with CD (29 women:29 men) and 28 patients with UC (9 women:19 men). The median age was 37 years. The treatment of the patients was ADA in 32 patients and IFX in 55 patients. The activity of the disease was reported by the clinician as high, moderate or inactive (remission). Drug levels and anti-drug antibodies were quantified before the next drug administration with the Promonitor ELISA assay (Progenika, Grifols SA) for Infliximab (IFX), anti-Infliximab (anti-IFX), Adalimumab (ADA) and anti-Adalimumab (anti -ADA). **Results**: Anti-drug antibodies were detected in 10 patients (12%): 7 patients under treatment with IFX (13% of all patients with IFX) and 3 patients under treatment with ADA (9% of all patients with ADA). The presence of anti-drug antibodies was associated with high activity in 5 patients (50%), moderate activity in 4 patients (40%) and inactive disease or in remission in 1 patient (10%). In the remaining patients, without development of anti-drug antibodies, the activity was high in 4 patients (5%), moderate in 45 patients (60%) and inactive or in remission in 27 patients (35%). The circulating levels of drug in patients without are:
a)
Infliximab (N=47)
High activity (N=3): 0,0 (0,0-0,2) ug/mL
Moderate activity (N=25): 3,2 (2,2-4,8) ug/mL
Remission (N=19): 3,4 (0,6-6,0) ug/mL
b)
Adalimumab (N=29)
High activity (N=1): 0,01 ug/mL
Moderate activity (N=20): 6,4 (4,1-9,1) ug/mL
Remission (N=8): 9 (4,9-16,2) ug/mL
Conclusions:
i) Anti-drug antibodies were present in 12% of patients on biological anti-TNF therapy. The percentage of immunogenicity (development of anti-drug antibodies) was similar for IFX (13%) and ADA (9%). ii) The presence of anti-drug antibodies is associated with high disease activity in patients. iii) In patients without antibodies; circulating serum levels of drugs were higher in those patients in remission versus moderate activity and high activity.

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A new biomarker of polyclonal B-cell hyperactivity in patients with systemic lupus erythematosus

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Background: Systemic Lupus Erythematosus (SLE) is a chronic systemic autoimmune disease with a variable disease course characterized by periods of remission and relapses. The clinician is faced with a shortage of adequate biomarkers to monitor the disease while those that are available do not always reflect the activity of the disease. In patients with LES there is a polyclonal hyperactivity of the B lymphocytes. So, the quantification of free light chains in serum and, more specifically, its sum (K+L) that we have defined as "(K+L) Index", could be as a potential biomarker of the patient's immune status. The objective of the study is to evaluate the utility of the (K+L) Index as biomarker of activity in patients with SLE. The following studies were carried out: i) compare the values of the (K+L) Index between healthy controls and patients with SLE, ii) compare the values of the (K+L) Index in the patients with SLE according to the activity of the disease, iii) correlate the values of the (K+L) Index with the biomarkers of activity C3, C4 and anti-DNA, iv) compare the values of the (K+L) Index in patients with SLE according to the positivity for anti-DNA antibodies. **Materials and methods:** A case-control study was performed in 57 healthy controls (age=45 years (37-51)) and 48 women diagnosed with SLE (age=36 years (29-40)). The degree of activity of the disease was assessed with the SLEDAI index: 24 patients with inactive disease (SLEDAI=0) and 24 patients with active disease (SLEDAI>0). The variables studied were: (K+L) Index, SLEDAI index and the biomarkers of SLE activity: C3, C4 and anti-DNA antibodies. The Mann-Whitney test was used for comparisons between quantitative variables and the Spearman correlation analysis to study the correlation between variables. **Results:**
Objective 1: The (K+L) Index was higher in patients with SLE vs. healthy controls: 45.34 (27.07-63.90) mg/L vs. 25.65 (19.93-29.26) mg/L, respectively ($p<0.0001$). **Objective 2:** The (K+L) Index was higher in patients with active disease (SLEDAI>0, n = 24) vs. disease in remission (SLEDAI=0, n = 24): 61.63

(44.23-66.45) mg/L vs. 34.07 (26.31-52.89) mg/L, respectively (p=0.002). **Objective 3:** A moderate correlation was obtained between the (K+L) Index and the complement C4 (rho=-0.495, p<0.0001) while no correlation was obtained with the complement C3 (rho=-0.150, p=0.3) and anti-DNA antibodies (rho=0.199, p=0.3). **Objective 4:** The (K+L) Index was higher in patients with positive anti-DNA antibodies (anti-DNA>30 U/mL, n=23) vs. negative anti-DNA antibodies (anti-DNA<30 /mL, n=25): 50.61 (43.89-63.90) mg/L vs. 27.28 (26.31-61.89) mg/L, respectively (p=0.018). **Conclusions**: The (K+L) Index in patients with active SLE is significantly higher compared to patients with SLE in remission and healthy controls. In addition, the (K+L) Index allows to differentiate between patients with positive and negative anti-DNA. The results suggest that the (K+L) Index would reflect the activity of B cells in patients with SLE and it would be a potential biomarker of activity to be included in the follow-up of patients with SLE.

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Performance of A New Third Generation TRAb Quantitative Assay* on Fullyautomated Immunoassay Analyzer

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Background: Hyperthyroidism in Graves' disease (GD) is caused by autoantibodies to the TSH receptor (TSHR), and quantitative assay of the TSHR autoantibody (TRAb) is widely used for diagnosis of GD and monitoring patients with GD. Fully-automated third generation TRAb quantitative assays utilizing monoclonal antibody M22 have been launched, but most of the on-market TRAb assays comprise a lyophilized TSHR-component which users need to reconstitute with liquid. We have developed a new third generation TRAb quantitative assay (ARCHITECT TRAb) by using a liquid (ready-to-use) TSHR-component on the fully-automated chemiluminescent immunoassay analyzer. **Objective:** To evaluate key performances of the newly developed prototype ARCHITECT TRAb assay. **Methods:** ARCHITECT TRAb reagent lots under development were used. The assay needs 50uL of sample, 6-point calibrators from 0 to 50 IU/L and 29 minutes of reaction time for first result. Key performances were evaluated according to CLSI or similar protocols on imprecision (20 days; 2 reagent lots, 4 analyzers), limit of quantitation (LoQ) (2 reagent lots, 3 analyzers), linearity (1 reagent lot, 1 analyzer), method comparison (95 graves' and 297 normal serum specimens; 3 reagent lots, 3 analyzers) with an on-market third generation TRAb assay; Roche Elecsys Anti-TSHR and reagent on-board drift (2 reagent lots, 2 analyzers). **Results:**

- Total imprecision: 8.1% to 11.4% (2.0 - 5.0 IU/L), 1.0% to 8.6% (5.0 - 50 IU/L)
- LoQ at 20% CV: 0.6 to 0.9 IU/L
- Linearity: 0.9 to 40.7 IU/L with read-out value shifts within +/- 0.3 IU/L (< 2.0 IU/L) or +/- 15% (≥ 2.0 IU/L) from linear regression
- Method comparison with an on-market third generation TRAb assay: Slope = 1.09 (passing bablock), r-value = 0.96
- Reagent on-board drift after one-time calibration: 7 to 14 days with read-out value shifts within +/- 0.3 IU/L (< 2.0 IU/L) or +/- 15% (≥ 2.0 IU/L)

Conclusion: The ARCHITECT TRAb assay demonstrated good precision, sensitivity, linearity and reagent on-board stability. Method comparison data showed acceptable agreement with an on-market third generation TRAb assay. The ARCHITECT TRAb assay is expected to improve efficiency of TRAb testing because of its improved usability by the read-to-use TSHR-component.
* Under development

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The diagnostic value of the AESKULISA PR3 sensitive & AESKULISA MPO in the EUVAS-cohort

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Objective: Anti-neutrophil-cytoplasmic-antibodies directed against proteinase-3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) are serological hallmarks of small vessel vasculitis, particularly granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). In a recent multicentre European-Vasculitis-Study-Group (EUVAS) evaluation, the performance of IIF was compared to that of various antigen-specific immunoassays. The aim was to evaluate the diagnostic accuracy of the third-generation antigen-specific immunoassays PR3-ANCA (AESKULISA-PR3-sensitive) and MPO-ANCA (AESKULISA-MPO) and to compare these data with the results from the other assay (Orgentec). *Methods*: 257 samples from the EUVAS cohort were tested for the presence of ANCA by PR3-ANCA ELISA (AESKULISA-PR3-sensitive) and MPO-ANCA ELISA (AESKULISA-MPO). Newly diagnosed GPA/MPA (n=66) patients and diseased controls (n=191): systemic lupus erythematosus (n=60), systemic sclerosis (n=10), rheumatoid arthritis (n=90), Scleroderma (n=11) and Sjögren's syndrome (n=30) were analyzed. *Results*: In AAV patients, ANCAs were detected with both methods in 56 cases; divergent results were obtained in only 1 patient sample. 191 patients with other rheumatic diseases were analyzed and only 13 vs 11 (AESKU/Orgentec) were positive for ANCA (SLE, sclerosis, RA, RA/RV). This study shows that the PR3- and MPO-ANCA ELISA are highly specific (93.2%/94.2%) and sensitive (85.9%/85.9%) in the detection of ANCA to identify AAV or conditions known to be associated ANCA. *Conclusions*: Our comparison of PR3- and MPO-ANCA ELISAs showed (i) a high diagnostic performance of these PR3- and MPO-ANCA ELISAs to discriminate AAV from disease controls. (ii) very good correlation between the other methods tested. In conclusion, these novel assays can be used as screening method for detection of ANCA-associated diseases.

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Effect of opiate abuse on miRNA 155 and 187 and inflammatory cytokines in chronic opiate dependent male addicts

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Background: Opioids can interfere with the immune system by participating in the function of the immune cells and causing modulation of innate and acquired immune responses. Recent studies indicate that the role of opioid receptors on immune function is complicated and works through various mechanisms. The study aimed to analyse the effect of opiate addiction on expression of miR187 and 155 in male chronic opiate addicts. **Methods:** The study included 46 chronic opiate dependents and 40 healthy control male subjects attending the de-addiction clinic at a non-government organization and a tertiary care centre. The study subjects were chronic opiate abusers (pure opium/ heroin) belonging to the age group 18 to 50 years. The subjects were analysed clinically for blood pressure. The venous blood samples were analysed for inflammatory cytokines IL-10, IL-6, TNF-alpha using ELISA and processed for extraction of miRNA. Kit based extraction of miR 187 and 155 was done (Qiagen) and c-DNA synthesized and stored at -80^{0}C. Relative gene expression of miR 187 and 155 was done by real time PCR and ΔCq values calculated using GAPDH as housekeeping gene. The data was analysed statistically by students' paired T test, Pearson's correlation and multiple regression analysis. **Results:** The study subjects had a mean age of 32±9.0 years (opiate dependents) and 33±7.34 years (controls). The diastolic blood pressure (DBP) was significantly reduced in the opiate dependent subjects (p=0.001). The serum levels of IL-10 and TNF-alpha were significantly raised in the opiate dependents as compared to controls (p=0002 and 0.03) whereas IL-6 was significantly reduced in opiate dependents (p=0.001). The ΔCqmiR155 (12.328±7.037, p=0.0311) and ΔCqmiR187 (15.242±2.361, p=0.0219) in opiate dependents were significantly higher than control subjects. Thus the relative gene expression of miR 155 is higher than miR187. Multiple regression analysis carried out with the pure opium dependents showed that ΔCqmiR155 was significantly correlated with IL-6 and was independent, significantly positively predicted by IL-6 (p=0.05) and negatively predicted by IL-10. In subjects dependent on heroin, ΔCqmiR155 was significantly correlated to IL-10 (p=0.004), dependency months (p=0.031). Further, multiple regression predicted ΔCqmiR155 independently (F=2.984, p=0.032) by IL-10, IL-6, dependency months, SBP and DBP with dependency months being the strongest predictor (p=0.009) and an associated memory loss (p=0.019). ΔCqmiR187 in pure opium dependent and heroin dependent subjects, there was a significant association with DBP (p=0.04) but non-significant association with IL-10, IL-6 and dependency months. **Conclusion:** The study results suggest that in chronic opiate addiction causes down regulation miR187 possibly acts via IL-10 axis inhibiting IL-6, promoting TNF-alpha. However, in heroin abusers, the miR155 expression may be enhanced with increasing dependency months, as in case of heroin abusers. Thus chronic opiate abusers are a unique group with significant immune imbalance expression profile which may be the basis of their tendencies of infection and cognitive decline.

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Validation of Alzheimer's Biomarkers: â-Amyloid 1-42 and Total Tau in CSF by Automated CLEIA on Lumipulse G 1200 Platform

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Background:
Guideline for Alzheimer's disease diagnosis (AD) suggests using AD biomarkers for the pre-symptomatic and symptomatic phases. Cerebrospinal fluid (CSF) level of β-amyloid 1-42 (Aβ-42) and total Tau proteins have been increasingly included in the diagnostic process of Alzheimer's disease. Aβ-42 is cleaved from amyloid precursor protein which ends up as aggregates in the brain, Aβ-42 plaque depositions are widely used to characterize AD. Analysis of Aβ-42 in CSF of AD patients shows significant reduction of Aβ-42 concentration. Tau is a neuronal protein which binds to microtubules in the neuronal axons. In healthy controls, levels of total Tau in CSF increase with age. Total tau levels are significantly enhanced in AD patients as compared with age-matched control subjects. Fujirebio (Fujirebio Inc., Japan) has developed fully automated chemiluminescence enzyme immunoassays (CLEIA) for analysis of Aβ-42 and total Tau in CSF. The purpose of this study is to evaluate the performance of these assays as per CLSI guidelines.
Method:
CSF Aβ-42 and total Tau are measured quantitatively by chemiluminescence enzyme immunoassay technology by a two-step immunoassay method on the LUMIPULSE G 1200 (Fujirebio Inc., Japan) using respective immunoreaction cartridges.
Results:

Validation Parameter	Experiment design	Results	
		Aβ 1-42	Total Tau
Precision	**Intra Assay:** 3 levels, 20 replicates each, 1 day	Average % CV= 1.43	Average % CV= 3.50
	Inter Assay: 3 levels, 1 replicate per day, 10 days	Average % CV= 1.66	Average % CV= 4.73
Accuracy	**- Abeta 42**: 5 proficiency samples **-Total Tau:** 3 kit controls	All samples within manufacturer's range Avg recovery: 96%	All samples within manufacturer's range Avg recovery: 96%
Analytical Measuring Range	5 levels of CSF spiked with recombinant protein, 4 replicates each, 1 day	14 to 2069 pg/mL, slope = 0.977	141 to 1919 pg/mL, slope = 1.025
Sensitivity (LLOQ)	5 levels, diluent spiked with recombinant protein, 5 replicates each per day, 5 days	14 pg/mL, CV = 10%	141 pg/mL , CV = 12%
Dilution Verification	Two samples diluted with diluent with 2 fold dilution up to X16, tested in duplicate	10X dilution acceptable ULOQ = 20,690 pg/mL	Dilution not allowed as per Package insert ULOQ = 1919 pg/mL
Length of Run	3 levels of QC, over 3 days at three time points (morning, afternoon and evening)	No significant change throughout the day	No significant change throughout the day
Reference Interval/ cut off	Not performed as samples not available	NA	NA

Conclusion:
Lumipulse G Aβ-42 and total Tau are robust quantitative assays and may meet the Clinical and Laboratory Standards Institute (CLSI) requirements. CSF Aβ-42 and Total tau could be proposed in clinical or drug trials as markers for AD according to the guideline.

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Validation of new Access 3rd International Standard Thyroid Stimulating Hormone (TSH) Assay and its Correlation with Access Hypersensitive TSH and Siemens Ultra TSH assays.

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Background:
TSH is a glycoprotein consisting of two non-covalently bound subunits; an alpha and beta subunit. TSH released from the anterior pituitary is the principle regulator of thyroid function by stimulating the synthesis and release of thyroid hormones. Clinical use of measurement of TSH is for the assessment of thyroid status. The sensitivity of the TSH assay has increased substantially over last several decades leading to the current 3rd generation TSH assays. Current TSH methods across platforms claim traceability to the WHO 2nd international reference standard 80/558, and thus assays are expected to be equivalent. Upon depletion of the current WHO 2nd standard, WHO released the3rd international standard (3rd IS) 81/565. Beckman Coulter has released a new Access TSH assay that is standardized to 3rd IS (Access 3rd IS). The objective of this study is to validate the Access 3rd IS and determines its combinability with both Beckman Access Hypersensitive (HS) TSH assay and with Siemens TSH 3 Ultra assay.
Method:
Precision, accuracy, linearity, sensitivity and reference range of the Access 3rd IS are evaluated following CLSI guidelines. A minimum of 30 native samples covering the AMR of the TSH 3rd IS assay (0.01 – 50.0 mIU/mL) were analyzed in parallel on all three methods. Analytical Measuring Ranges (AMR) and Reference Ranges (RR) are, 0.01 – 50.0 mIU/L and 0.45 – 5.33 mIU/L, respectively, on the Access TSH 3rd IS; 0.02 – 100.0 mIU/mL and 0.34 – 5.60 mIU/mL, respectively, on Access the HS TSH; and 0.008 – 150 mIU/mL and 0.55 – 4.78 mIU/mL respectively, on Centaur TSH3-ultra.
Results:

Method Comparison, TSH	N	Range (mIU/mL)	Slope	Intercept	Corr Coef., R
Access HS Vs. Access 3rd IS	32	0.540 – 33.970 (AMR)	0.942	0.151	0.995
	23	0.540 – 7.800 (RR)	1.014	0.017	0.988
Access HS Vs. Siemens 3 Ultra	32	0.540 – 33.970 (AMR)	1.012	0.007	0.995
	23	0.540 – 7.800 (RR)	0.970	0.074	0.991
Siemens 3 Ultra Vs. Access 3rd IS	32	0.533 – 35.782 (AMR)	0.931	0.145	0.996
	23	0.533 – 7.470 (RR)	1.045	-0.060	0.994

Conclusion:
The Access 3rd IS assay was successfully validated and demonstrated acceptable performance as per CLSI guidelines. Also, the assay is equivalent with both Access Hypersensitive (HS) TSH assay and with Siemens TSH 3 Ultra assay, especially at the lower end of the AMR.

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New technology for improvement of sensitivity and specificity of IVD-Tests

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Background:
Some test systems are lacking of high sensitivity and specificity which otherwise enable early and accurate diagnosis and treatment being beneficial for the course of the disease. This is known for Graves' disease (GD), which is caused by thyroid stimulating immunoglobulins (TSI) directed against the thyrotropin receptor (TSHR). Current TSH or M22 displacement assays (TDA) quantify indirectly all TSHR autoantibodies (Ab). We developed a direct epitope recognition assay (DERA) for sensitive detection of TSI in which the extracted capture human(h) wtTSHR chimera is anchored on microtiter plates (sTRAb) and stimulatory epitope binding TSI are bridging to a soluble signalling chimeric extracellular domain (ECD) of the hTSHR. Here we show a Bridge assay in which a novel truncated capture hTSHR can be anchored on paramagnetic microbeads (PMB).
Methods:
PMB DERA is performed by bridge technology were an antibody anchors to PMB the solitary chimeric ECD of hTSHR-rLH/CG receptor fused with a secretory protein. The TSI binds with one arm to the immobilised chimeric capture hTSHR, and the second arm bridges to a chimeric hTSHR ECD fused with secretory alkaline phosphatase or HRP. Applying chemiluminescence substrate sTRAb were quantified using a plate luminometer. The PMB assay was performed manually with a 12-tube magnet. For further development, we used PMB on microtiter plates.
Results:
The chimeric ECD of hTSHR-rLH/CG receptor shows a functionality/half life of 12/15 days at 4°C. ROC analysis of sTRAB DERA with 274 GD positive and 325 GD negative show a criterion for positivity > 0.54 IU/L with a sensitivity of 99.8 % and a specificity of 99.1 % in the assay. The PMB assay performed manually with a 12-tube magnet enabled ROC analysis of 190 samples (136 GD positive, 54 GD negative) and shows a criterion for positivity > 1.5 IU/L with a sensitivity

of 94.1% and a specificity of 98.2%. In comparison the sensitivity/specificity/cut off from the following tests: Roche Elecsys, cobas anti-TSHR 97.0%/99.0%/>1.75 IU/L; Thermo Brahms Kryptor: 96.3%/98.1%/>1.80 IU/L; Immunite 2000/XPi TSI: 98.3%/99.7%/>0.55 IU/L; sTRAb-DERA: 99.8%/99.1%/>0.54 IU/L; PMB-Bridge-assay 94.1%/98.2%/>1.50 IU/L. Further preliminary experiments with PMB performed on microtiter plates using the signaling hTSHR-ECD-HRP as detection receptor show a calibration curve reaching a clinical cut off of 0.2 IU/L. These results on microtiter plates are a basis for the development of automation for the PMB Bridge assay.
Conclusion:
For the first time an immobilized chimeric hTSHR ECD is shown to be excellently used on PBM in a Bridge assay which as a further innovation works with two secreted TSHR ECDs. Further improvement of technical statistics (sensitivity and specificity) is expected by transferring this prototype to fully automatic systems. Together with the good stability data automatization will improve sensitivity and specificity in comparison to other current IVD-assays by a novel technology. Further, we wish to apply this method for other autoimmune diseases such as diabetes type 1 in future cooperative studies. Also in diabetes type 1, early diagnosis and treatment will lead to less severe course of the disease.

A-370

Multiplexed, Isotype-specific Ultrasensitive Research-use Bridging Serology Assays for Detection of Autoimmune Reactivities

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Background: Autoimmune diseases affect over 50 million Americans and have rapidly rising incidence. The presence of multiple specific autoantibodies can often predict disease onset in at-risk individuals [e.g. type 1 diabetes (T1D), systemic lupus erythematosus, celiac disease], and assist in distinguishing disorders with similar clinical features (e.g. T1D versus type 2 diabetes). In an ongoing study to assess the feasibility of developing highly sensitive and specific multiplexed antigen-bridging serology panels for detection of autoimmune biomarkers, the MSD® MULTI-ARRAY technology is being used to produce research-use assays for detection of T1D, other organ-specific autoimmune disease, and connective tissue disorder biomarkers. First generation multiplexed assay panels developed to detect T1D autoantibodies to glutamic acid decarboxylase (GADA), insulinoma 2 (IA2A), and insulin (IAA), as well as markers relevant to celiac disease, autoimmune gastritis, and thyroid disease, were previously tested using assay proficiency evaluation samples from the Islet Autoantibody Standardization Program. In those studies, the MSD T1D-relevant assays performed comparably to existing assays, with the advantages of low sample requirements for multiplexed detection (less than 25 µL needed for detection of up to 10 biomarkers), high throughput, and no radioactivity [Mathew et al. (2016) Diabetes 65(Supplement 1):A431-A440, 1673P]. In the current phase of the project, next generation assays have been developed using MSD's ultrasensitive assay format to enhance multiplexed detection of autoimmune disease-related reactivities with the additional capability to discern autoantibody isotypes. **Objective:** The objective of the study was to compare performance of the first and second generation MSD bridging serology assay panels in a 96-well plate, high throughput format. The assays were assessed using commercially purchased T1D and presumably normal individual sera. The multiplexing capability enabled simultaneous quantitative measurement of T1D and comorbid disease markers. The second generation assay was formatted for detection of IgG reactivities, but can easily be formatted for detection of other antibody isotypes (IgA, IgM). **Results:** Preliminary data show that assay sensitivities/specificities improve from 65%/100% to 91%/96% (IAA detection), 57%/100% to 70%/92% (GADA detection), and 35%/95% to 48%/96% (IA2A detection) when comparing the first and second generation assays, respectively. Furthermore, ultrasensitive assays were included for detection of pro-insulin autoantibodies (pro-IAA) and zinc transporter 8 (ZnT8) that demonstrated initial specificity/sensitivity values of 96%/100% and 22%/92%, respectively. Two or more T1D reactivities were detected in eighteen of the twenty-four T1D samples tested and in one of the twenty-five normal samples screened. **Conclusion:** The sample-sparing ultrasensitive multiplexed MSD autoantibody assays significantly enhance the ability to identify samples containing multiple autoimmune reactivities, with the capability to distinguish specific antibody isotypes, while retaining the high specificity of the bridging serology assay format. The reported research was supported by the NIAID of the NIH under Award #1 U24 AI 118660. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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A combination of histo-immunoprecipitation, mass spectrometry and recombinant cell-based indirect immunofluorescence identifies novel neural autoantigens

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Background: Indirect immunofluorescence (IF) using cultivated (recombinant) human cells and cryosections of mammalian tissues as antigenic substrates is still the gold standard for the screening of autoantibodies. He we report on the combination of IF with histo-immunoprecipitation (HIP) and mass spectrometry to enable identification of autoantigens in the growing field of neuroimmunology. **Methods:** Sera and CSF of patients with neurological symptoms were comprehensively investigated for the presence of anti-neural IgG autoantibodies by immunoblot and IFA including 35 established recombinant antigen substrates. Samples staining neural tissue sections but devoid of reactivity with established monospecific test substrates were subjected to HIP, employing cerebellum and hippocampus cryosections, followed by fractionated extraction of the immunocomplexes and PAGE/MALDI analysis. Samples with autoantibodies against NMDAR and DPPX were used as positive controls. The identified antigen candidates were recombinantly expressed in HEK293 and used as substrates for IF. **Results:** Most samples used in this study produced speckled, granular or homogenous staining of the hippocampal and cerebellar molecular and granular layers. Others specifically stained cerebellar Purkinje cells. Up to now, eighteen different autoantigens could be identified by this approach, among them ITPR1, NCDN, ATP1A3 and flotillin as novel autoantigens. **Conclusion:** Based on their reaction with autoantibodies in the native tissue environment HIP/PAGE/MALDI in combination with IF is able to isolate, identify and verify autoantigens. The RC-IFA substrates resulting from this approach can readily be used in serology. The diagnostic value of the novel autoantigens has to be evaluated in cohort studies.

A-372

Evaluation of Crithidia luciliae IFT can be reliably automated with EUROPattern

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Background: Automation of immunofluorescence microscopy resolves general limitations of IIFT in autoimmune diagnostics, such as high work load and subjectivity of interpretation. An additional software concept of EUROPattern-Suite - a combination of automated microscope and classification software - now enables automated evaluation of anti-dsDNA in Crithidia luciliae-based IIFT (CLIFT). Anti-dsDNA are primarily present in systemic lupus erythematosus (SLE) with a varying prevalence of 20 % to 90 %. **Methods:** 60 samples from clinically confirmed SLE patients and 117 samples from patients with other autoimmune or infectious diseases were tested for anti-dsDNA using CLIFT. Results, automatically retrieved by the classification software, were compared to visual fluorescence interpretation performed by two independent experts on the computer screen. **Results:** Visual evaluation of CLIFT revealed 27/60 anti-dsDNA positive SLE patients (prevalence 45 %). 26 of them were also classified as positive by the EUROPattern Suite, in total the system identified 28 positive SLE patients. Of 33 SLE patients and 115 control patients evaluated as negative by the experts, the software also classified 31 and 112 patients as negative **Conclusion:** With respect to visual IIFT interpretation, the EUROPattern Suite was 96.3 % sensitive and 96.6 % specific and performed in major agreement with the experts, reaching an accordance of 96.6 %. This high agreement is a precondition for reliable application of the software in routine diagnostics to support the reduction of human workload and the standardization of fluorescence interpretation without a loss in quality.

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Computer-aided immunofluorescence microscopy of recombinant cell-based IIFT in neurological diagnostics

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Background: Several target antigens have been identified recently as being associated with autoimmune neurological diseases. Multiparametric testing of most frequent neuronal parameters using recombinant cell-based BIOCHIP mosaics for IIF analysis is appropriate to support rapid differential diagnosis. Evaluation of these IIFT can now be semi-automated using the EUROPattern Suite, an established system for computer-aided immunofluorescence microscopy (CAIFM). **Methods:** Antibody reactivities against NMDA and AMPA receptors, CASPR2, LGI1, GABABR, DPPX and aquaporin-4 were assessed in 880 incubations of serially diluted samples using the Autoimmune Encephalitis Mosaic 6 and the Anti-Aquaporin-4 IIFT (Euroimmun AG, Luebeck). Two evaluation methods were compared: visual inspection of substrates at the microscope and examination of corresponding digital images, automatically taken by the EUROPattern microscope and represented in a clearly arranged configuration at the computer screen. Borderline fluorescence signals at the microscope were excluded to avoid superposition of results by inter-reader deviations. **Results:** One high-resolution image per substrate (BIOCHIP) of the mosaic is taken within an average time of seven seconds using the 10x objective. Propidium iodide (PI) counterstaining supports sharp autofocusing of the cells and is presented to the user for visual verification of the focal plane. This is important for negative samples which do not reveal any fluorescence signal. In this study, immunofluorescence evaluation at the computer screen correlated with classical microscopic evaluation to 100 %. **Conclusion:** On-screen evaluation of digital images provides equal diagnostic quality compared to visual microscopic evaluation. Concomitantly, automated focusing and image acquisition by the EUROPattern microscope facilitates the evaluation. all images regarding one patient are clearly arranged on the screen. Results are directly entered within the graphical user interface of the software EUROLabOffice, developed for digital laboratory data management and archiving.

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Method validation of ANA testing by Indirect fluorescence assay (IFA) using Helios automated analyzer vs. enzyme based immunoassay (EIA)

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Background: The antinuclear antibodies (ANA) test is used to aid in the diagnosis of autoimmune diseases, including systemic lupus erythematosus, Sjogren's syndrome, scleroderma, mixed connective tissue diseases, and systemic rheumatic diseases. A positive ANA screen could be subsequently reflexed to a more specific antibody test to further assess the presence of commonly seen ANA antibodies (such as Smith, RNP, SS-A, SS-B, Scl70, and Jo-1), or indirect immunofluorescence assay (IFA) can be performed with reflex to titer . Our current screening method is an enzyme based immunoassay (EIA) Immco ImmuLisa Enhanced ANA screen performed on the Dynex DSX platform. In this study, we sought to compare our current ANA EIA test to the IFA based method using Helios automated analyzer with AESKUSLIDES ANA HEp-2-Gamma assay. Additionally, we also compared the manual ANA titers (Zeus reagent) and interpretation to the Helios automated analyzer, equipped with estimated endpoint titer and pattern recognition software for ANA HEp-2 IFA. Methods: This study examined serum samples from 164 patients for ANA screen performed by EIA on the Dynex DSX platform and by IFA on the Helios automated platform. A total of 101 patient serum samples were evaluated for method comparison between manual IFA and Helios automated IFA for pattern recognition and titer reading. A 1:80 titer was used to distinguish absence or presence of fluorescence on both platforms. Further pattern interpretation was compared using between Helios pattern recognition software for ANA HEp2IFA patterns and the gold standard manual interpretation. **Results:** Method comparison between the EIA screening method and the Helios IFA method demonstrated 95.4% positive agreement and 89.6% negative agreement, giving a total agreement of 92.7%. Of the 164 samples, four samples that were screened positive by EIA but negative by Helios IFA were further confirmed to be negative by ANA IFA titer. The fourth sample was a weak positive therefore is the only true discrepant for the positive EIA sample. Qualitative correlation for reflex titer was performed comparing the gold standard manual method and Helios IFA titer method, using 1:80 was used to distinguish between absence or presence of fluorescence. There was a 98.7% positive agreement and 100% negative agreement between the two methods, which gave a total agreement of 99%. Helios IFA pattern recognition ability scored a 95.8% agreement in comparison to the manual interpretation. **Conclusion:** The correlation between the EIA and the Helios IFA methods for ANA screening and titer both showed excellent concordance. For those that were not in agreement during the initial screen but were further confirmed by ANA titer showed that the Helios IFA is by far a more sensitive method compared to EIA. Even though the Helios IFA titer pattern recognition is currently not FDA approved, it showed an excellent agreement to the manual gold standard method. Overall, the Helios platform is acceptable for performing ANA screen test and reflex titer confirmation in.

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Atypical ANCA Testing with an Expanded ANCA Specificity Profile

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BACKGROUND: Antibodies to Neutrophil Cytoplasmic Antigens (ANCAs) are associated with a variety of autoimmune diseases. C-ANCAs and P-ANCAs, when documented for PR3 or MPO specificity are important to the diagnosis and classification of autoimmune vasculitis. Standard testing involves screening for ANCAs by IFA [IIF], followed by a quantitative EIA method for anti-PR3 & MPO antibody levels. IFA ANCAs negative for both PR3 and MPO, if an ANA cross-reaction is excluded, have been termed an "Atypical ANCA". We have used a 6 test Expanded ANCA Specificity EIA profile (Euroimmun) which includes testing for anti- MPO, PR3, Lactoferrin, Elastase, BPI, and Cathepsin-G antibodies, resulting in detection of a variety of ANCA specificities, and a significant decrease in the number of "Atypical ANCA" reports. **METHOD:** All samples for ANCA testing are screened by IFA (INOVA) on both ethanol and formalin-fixed slides, tested for anti-MPO & PR3 levels (INOVA EIA) and for ANA (Euroimmun HEp-2 IFA). Samples showing an IFA-ANCA positive for anti-PR3 or MPO specificity are reported with pattern and titer. Samples showing an IFA-ANCA, negative for both PR3 and MPO specificity, are tested by the Expanded ANCA EIA profile. **RESULTS:** Results from a recent 24-month period show 998 [85%] of 1170 samples to be negative, 172 (15%) positive for ANCA. 59 (34% of positives) showed either PR3 or MPO ANCA; 62 (36% of positives) were deemed "Atypical ANCA" as an antigen specificity was not evident. Use of the Expanded 6-test ANCA profile showed 51 cases (30% of IFA-positive ANCAs) to have specificity for one or more of the additional antigens in the expanded profile. These results were sometimes crucial to establishing a specific diagnosis, and served to clarify an otherwise "Atypical ANCA". BPI-ANCA specificity was an isolated finding in 24 cases, each showing a C-ANCA pattern, and was often associated with severe infection. Elastase-ANCA [4 cases] occasionally correlated with Cocaine use, and Lactoferrin-ANCA [8 cases] occasionally with inflammatory bowel disease. Cathepsin-G ANCA was noted in three (3) cases. Multiple ANCA specificities were noted in twelve (12) cases, eleven of which included at least one of the "new" ANCA specificities. **DISCUSSION:** Fluorescent microscopic [IFA;IIF] detection of ANCAs is a global method showing visual evidence of patient antibody to a wide spectrum of neutrophil cytoplasmic and nuclear antigens, comparable to IFA detection of autoantibodies in HEp-2 cells. Diagnostically valuable information is gained by testing for antibody specificity in both situations—ENA profile follow-up to a positive ANA, and an expanded antigen-specific profile following a positive ANCA result. Diagnostic information is limited when limiting ANCA follow-up testing to only MPO & PR3 specificities, and terming all other ANCAs as "Atypical". ANCA antigen-specificity proved more significant than ANCA IFA patterns. **CONCLUSION:** Routine use of an Expanded ANCA Profile will further clarify and expand diagnostic utility of ANCA testing--similar to follow-up testing of an IFA-positive antinuclear antibody (ANA) with an expanded ENA profile.

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Sensitive and multiplex detection of anti-islet cell autoantibodies for type 1 diabetes (T1D) diagnostics and risk assessments

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Background: Clinical detection of anti-islet cell autoantibodies (autoAb) for type 1 diabetes (T1D) largely relies on radioimmunoassays (RIA). However, use of hazardous radioactive substance has limited its use to very few centralized clinical laboratories due to complicated waste disposal and regulatory processes. We expect a T1D autoAb assay that do not rely on radioactive materials could efficiently aid in decentralizing T1D testing and make the tests more affordable for wide arrays of patients in

need. **Methods:** Here we described a multiplex assay to quantify three major anti-islet cell autoAb, namely anti-glutamic acid decarboxylase (anti-GAD65), anti-islet antigen 2 (anti-IA2) and anti-insulin (anti-INS), in 1 µL sample. The underlying technology termed Antibody Detection by agglutination-PCR (ADAP) uses a ligation-based signal generation mechanism to convert antibody identities into PCR-amplifiable DNA barcodes, which allows preservation of antigen conformation, exponential amplification of DNA amplicons and DNA barcoding. These favorable attributes of ADAP lead to highly sensitive and multiplex detection of conformational sensitive T1D autoAb in a single assay. **Results:** We have validated the ADAP T1D assay in 6 clinical cohorts from diverse clinical centers (>400 unique samples). The data indicates the ADAP T1D assays share similar clinical accuracy in several blinded cohorts. The clinical sensitivity and specificity ranges from 90%-100%. Moreover, the signal intensities between ADAP and RIA correlates well. The coefficient of correlation (R) ranges from 0.85-0.93 for three anti-islet cell autoAb tested. In addition, we have also shown promise to detect T1D autoAb in alternative sample types such as whole blood and dried blood spot samples, which could promote the point-of-care (POC) testing and population-wide screening of T1D risks. The signal correlation between serum/whole blood and serum/dried blood spot are 0.87-0.98. **Conclusion:** We believe the data demonstrate ADAP T1D assay is as accurate as RIA assays in terms of clinical accuracy and autoAb titer determination. Importantly, the removal of radioactive reagents and capabilities to detect multiple anti-islet cell autoAb in a single assay could favorably ease clinical laboratory practice in T1D testing.

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Validation of a quantitative method for fecal zonulin

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Background: Zonulin is a human protein that appears to be the primary regulator of tight junctions between epithelial cells, including the epithelia of the intestinal tract. Zonulin binds to specific receptors on the surface of intestinal epithelia and triggers a cascade of biochemical events which induces tight junction disassembly and a subsequent increase in paracellular permeability of the intestinal epithelia. This increased permeability allows the influx of macromolecules from the intestinal lumen which invoke immune responses.[1] Our goal was to validate a method for the reliable measurement of zonulin in feces. Increased levels of serum zonulin antigen are found in several autoimmune disorders, including celiac disease, insulin-dependent diabetes, multiple sclerosis, and rheumatoid arthritis. Fecal levels of zonulin have not been as extensively studied, but increased fecal zonulin levels have been reported in patients with metabolic syndrome.[2] **Methods:** The ImmunDiagnostik Zonulin assay is based on a competitive enzyme linked immunosorbent assay (ELISA). A biotinylated zonulin tracer is added to samples, standards, and controls, which are subsequently transferred to a microtiter plate coated with polyclonal anti-zonulin antibodies. During incubation, the free zonulin in the sample competes with the biotinylated zonulin tracer for the polyclonal anti-zonulin antibodies immobilized in the microwells. Unbound components are removed by washing, and peroxidase-labeled streptavidin binds to the biotinylated tracer. After a second wash step, the substrate trimethylbenzidine is added to evoke a colorimetric response, and an acid solution stops the reaction. The optical density of standards, controls, and patient samples read at 450 nm is inversely proportional to the zonulin concentration. Analytical precision, linearity, recovery, limit of detection, and accuracy of this method were assessed, and sample stability was determined in freshly-collected fecal samples. Fecal samples provided by ambulatory adult volunteers (n=47) with no reported gastrointestinal maladies or autoimmune disorders were used to determine a reference interval using EP Evaluator (Build 11.3). **Results:** The intra-assay and total imprecision coefficients of variation (CV_W and CV_T) (n=20) of zonulin in fecal samples was determined as 5.7% and 13.9% at 35.0 ng/mL, and 6.7% and 15.4% at 60.9 ng/mL. Serial dilution of a fecal sample with increased zonulin demonstrated linearity (n=10) as 13.0-209.0 ng/mL, with recovery of 95.4%-106.2%. The limit of detection was determined to be 5.25 ng/mL. Adequate stability of zonulin in fecal samples was demonstrated for 11 days stored at 2-8°C or -20°C. Non-parametric statistical assessment of the data distribution showed that the upper limit of the reference interval was 89.0 ng/mL (90% confidence interval: 80.1 – 97.9 ng/mL). **Conclusion:** This ELISA method for fecal zonulin has been validated to be analytically precise and accurate, while providing a robust analytical range. Sample suitability has been established for the expedited temperature-controlled transportation of fecal samples from remote locations to a central laboratory for analysis. Further research is warranted to determine if a single measurement of fecal zonulin is associated with abnormal results for the established lactulose-mannitol test for paracellular permeability.

References: 1) *Practical Laboratory Medicine.* 2017;9:39–44. 2) *International Journal of Molecular Sciences*. 2017;18(3):582.

A-378

Evaluation of the HELIOS automated immunofluorescence processor/reader for ANCA and anti-DNA testing

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BACKGROUND

Anti-neutrophil cytoplasmic antibody (ANCA) testing and anti-double stranded DNA antibody (anti-DNA) testing are often performed by immunofluorescence assays (IFA). The IFA procedure is labor intensive and requires subjective interpretations by trained technologists. Automated slide processors and separate, automated image analysis microscopes have been developed to address these IFA shortcomings. These dual platform systems require operator intervention to transfer slides from the processor to the reader. The AESKU HELIOS combines the processing and computer assisted image acquisition and analysis onto a single platform. Slides do not have to be transferred to a separate image acquiring microscope after processing, as both processes occur on the same instrument. Last year at this meeting we presented our evaluation of the HELIOS for anti nuclear antibody testing. In this study, we compared the HELIOS to manual IFA for ANCA and anti-DNA testing in a large community teaching hospital.

METHODS

For both ANCA and anti-DNA testing, we compared three different processing/reading methods. Method A- Automated Slide processing and automated interpretation on the HELIOS. Method B- Automated slide processing on the HELIOS; manual reading of the image on the computer by 2 independent, experienced readers Method C- Manual slide processing and microscopic evaluation by the readers. For the ANCA study, we analyzed 135 specimens from patients with autoimmune associated vasculitis, 120 from patients with documented ANCA and 375 from patients with other autoimmune and infectious diseases. All 630 specimens were tested at a 1:20 dilution on ethanol and formalin fixed neutrophils by the three different methods. For the anti-DNA study, we analyzed 297 specimens from patients with SLE and 479 from patients with other autoimmune and infectious diseases. All 776 specimens were tested on *Crithidia lucliaeluciliae* slides at a 1:10 dilution using the three different methods.

RESULTS

ANCA- On ethanol fixed slides overall positive and negative agreement of all method comparisons and both readers ranged from 89.4-96.8%. Pattern agreements for all comparisons and readers ranged from 77.8-89.2% with the highest agreements between methods B and C; both methods of which relied on manual interpretation. On formalin fixed slides, overall positive and negative agreement between all methods and both readers ranged from 77.6-95.9%. Pattern agreements for all comparisons and readers ranged from 69.7-90.8% with the highest agreements between methods A and B. Anti-DNA- Overall agreements between all methods and both readers ranged from 80.3.7-97.5%, Positive agreements for all comparisons ranged from 44.8-91% and negative agreements were 83.9-98.3%. The highest agreements were between methods B and C; both methods of which relied on manual interpretation.

CONCLUSION

The HELIOS automated slide processor/reader provides positive and negative qualitative results similar to manual processing and reading for ANCA and anti-DNA testing. No subjective difference was found between slides prepared manually and slides prepared on the automated HELIOS platform for either assay. Review of the HELIOS ANCA pattern interpretations by a trained reader will ensure that appropriate patient results are reported.

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Development of functional assays for complement components C1q, C2, C3 and C5.

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Complement proteins form part of the innate immune system. Deficiencies in the complement system can manifest as a failure to mount an effective immune response or in autoimmune conditions. Protein titer measurements in complement deficient patients can be misleading and functional tests may provide additional information. CH50 is a commonly used test to determine activity of the classical complement pathway. Abnormal results can indicate a problem with the production or consumption of one or more of the proteins in the classical complement pathway. Whilst useful, the measurement of CH50 does not help in identifying the specific deficiency. Here we describe simple adaptations to the CH50 Optilite® assay that can be performed on the Optilite analyser (The Binding Site Group Ltd., Birmingham, UK) to determine specific complement deficiencies. Mixing patient sera with sera depleted of a single known complement protein can de-

termine whether that functional protein is present or absent in the patient sample. Commercially available C2-depleted serum and sera from 2 healthy individuals were used to demonstrate the principle of the method. Undetectable CH50 activity was reported in the C2-depleted serum and CH50 results were 65.0 and 61.0 U/mL in healthy serum samples (normal CH50 reference range in serum: 41.68-95.06 U/mL). Mixing C2-depleted serum with sera from the healthy individuals (ratio 1:1) rescued CH50 activity (42.8 and 46.6 U/mL) indicating the presence of functional C2 in the healthy sera. Within- and between-sample variability was assessed for C1q, C2, C3 and C5-depleted serum. Within-sample variability was assessed by measuring CH50 activity in 10 replicates of a single premixed sample and between-sample variability was assessed by measuring CH50 activity in 10 individual healthy serum samples mixed 1:1 with complement-depleted serum. The within-sample coefficients of variation (CVs) ranged from 0.41%-2.53% and between-sample CVs ranged from 7.29%-11.68%. To validate the utility of this method in patient sera, CH50 activity was used to confirm a C2 deficiency in two patients. An undetectable C2 concentration (measured using Human Complement C2 SPAPLUS® assay, The Binding Site Group Ltd., Birmingham, UK) was reported in patient 1, who had a confirmed homozygous deficiency. Serum from patient 1 was combined with C2-depleted serum (ratio 1:1) and C5-depleted serum (ratio 1:1); CH50 assay results were undetectable and 27.9 U/mL, respectively. Thus the adapted method detected the absence of functional C2 and presence of functional C5 in the patient sample. A C2 concentration of 10.7 mg/L was reported for patient 2 (normal reference range: 18.7-44.0 mg/L). Consistent with this, when patient 2 serum was mixed with C2-depleted serum (ratio 1:1), CH50 activity was still below the normal reference interval (18.7 U/mL). In conclusion, a simple adaptation to the Optilite CH50 assay, mixing patient samples with commercially available complement-depleted serum, provides a simple method to detect absent or defective complement proteins.

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Antibodies against neo-epitopes of microbial and human transglutaminases

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Objectives and study: Microbial transglutaminase (mTg) and human tissue Tg (tTg) form complexes with gliadin peptides, thus posttranslating and modifying gliadin to present neo-epitopes. **The aims** were to test the diagnostic performance of antibodies against both non-complexed and complexed forms of both transglutaminases in children with celiac disease, compared with disease controls and to correlate antibodies' levels to the degree of intestinal atrophy. **Methods:** Serum samples, at day of intestinal biopsy, were collected from 350 children with celiac disease (mean age 7.4 years) and 215 disease controls (mean age 10.2 years) and tested using the following ELISAs detecting IgA, IgG or both IgA+IgG combined (Check): tTG (for in house research use only), *AESKULISA*®s tTg New Generation (tTg neo-epitope (tTg-neo)) and mTg neo-epitope (mTg-neo, RUO). Results were correlated to the degree of intestinal injury, using the revised Marsh criteria. **Results:** mTg-neo Check had the highest sensitivity and tTg IgA the highest specificity. Comparing the different correlations between antibodies' isotypes, the tTg Check (r=0.7889, p<0.0001) and tTg-neo Check (r=0.7544, p<0.0001) as well as tTg IgA and tTg-neo IgA (r= 0.7571 and r= 0.7279, p<0.0001 respectively) were the best indicators of intestinal damage in CD. **Conclusion**: It is suggested that the combination of tTg-neo IgA/IgG antibodies should be recommended as a first line screening test for CD in children. The tTg and tTg-neo assays show similar diagnostic performance and are recommended as good screening tests for CD in children. mTg-neo IgG presents a new serological biomarker for celiac disease.

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IgG, IgA and IgM responses to pneumococcal polysaccharide vaccination (Pneumovax®) in a normal healthy adult population

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Background: Assessment of the response to pneumococcal polysaccharide vaccination (Pneumovax®, PPV) may help identify antibody deficient patients who are most at risk of infection. A complete response to PPV would include production of antigen-specific IgG, IgA and IgM antibodies, but currently immunological investigation only requires measurement of the IgG isotype. In this study, we determined the concentration ranges of pneumococcal IgG, IgG2, IgA and IgM in response to Pneumovax in a normal healthy adult population using VaccZyme™ anti-PCP ELISAs. **Methods:** We determined pneumococcal IgG, IgG2, IgA and IgM concentrations pre-vaccination, at 4-6 weeks and at 6 years post- Pneumovax vaccination in 79 normal healthy Prevnar® naïve adults (41 males, 38 females, median age 44 years, range 22-66 years) using VaccZyme PCP IgG, IgG2, IgA and IgM ELISAs (The Binding Site Group Ltd., Birmingham, UK). **Results:** The median pre- and post-vaccination concentrations for PPV IgG, IgG2, IgA and IgM are presented in Table 1. Four to six weeks post-vaccination there was a (median, range) 6-fold (2-24) increase in PPV IgM, 18-fold (4-74) increase in IgA, 9-fold (2-19) increase in IgG and 8-fold (1-20) increase in IgG2. Fold increases were significant in all age ranges (p<0.0001). The median concentrations 6 years after vaccination were significantly elevated for all age groups compared to the pre-vaccination concentration for PPV IgA, IgG and IgG2 isotypes (p<0.001 - p<0.02) but not for PPV IgM (P=0.1-0.7). The PPV IgM and IgA, but not IgG responses were influenced by age. **Conclusion:** The definition of normal adult reference ranges will aid clinical interpretation of a deficient polysaccharide response in those suspected of antibody deficiency.

Median pre- and post- PPV vaccination concentrations (and 95% CI) for PPV IgG, IgG2, IgA and IgM

	Vaccination pre/post		
	Pre	4-6 weeks post	6 months post
IgG (mg/L	43 (11-186)	375 (77-1238)	151 (31-1073)
IgG2 (mg/L)	18 (4-120)	141 (25-573)	59 (10-473)
IgA (U/mL)	22 (6-102)	369 (78-1802)	85 (19-279)
IgM (U/mL)	53 (16-168)	315 (60-1133)	54 (17-128)

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Development of a 3 step screening procedure for the measurement of pneumococcal antibodies

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Background: Currently there are two different methods by which pneumococcal serotype antibodies may be quantified; measurement of the summation of all serotype specific pneumococcal antibodies or measurement of the antibodies individually, usually in a multiplex format. Here, an algorithm for the measurement of pneumococcal antibodies that screens using summation and reflexes to individual serotype analysis is presented. **Methods:** Serum samples were obtained from 1510 individuals referred to the Mayo Clinic. Serotype antibody concentrations for all 23 serotypes were available from 1264 individuals (M:F 499:765; median age 38 years, range 2-105). For validation of the algorithm, normal healthy volunteers (n=89; M:F 44:45; median age 44 years, range 20-66) and CVID patients (n=36; M:F 14:22; median age 39 years, range 14-74) post Pneumovax®23 vaccination were analysed. Total pneumococcal IgG antibodies was measured using a commercially available ELISA kit (VaccZyme™ Pneumococcal capsular polysaccharide (PCP) IgG ELISAs, The Binding Site Group Limited, UK, measuring range 3.3-270 mg/L) and Pneumococcal antibody serotype analysis was performed for 23 antibodies using a laboratory defined test based on Luminex technology. **Results:** Cut-offs of 9.7 mg/L and 270 mg/L in the PCP IgG ELISA were identified based on high confidence that either an antibody deficiency (<9.7 mg/L) or healthy response (>270 mg/L) would be identified by both methods. The agreement between both methods at each cut off was >98%. Samples with a concentration between 9.7mg/L and 270mg/L would be candidates for reflexing to pneumococcal serotyping. To further "screen" these antibodies using the simple PCP IgG ELISA, two further indeterminate cut offs at 40 and 180 mg/L were established. At these cut-offs, agreement between the total and serotyping assays was decreased (82% and 95% respectively). However, given the still significant agreement, our algorithm proposes that clinician discussion/clinical information should form part of the decision making process for reflexing to serotyping. Samples with a pneumococcal antibody concentration between the indeterminate ranges (e.g. 40-180 mg/L) would be automatic candidates for pneumococcal serotyping analysis which would account for 44% of total samples (554/1264). In order to validate use of the total PCP assay as a screening test prior to serotype-specific testing, the proposed cut-offs were applied to a cohort of healthy individuals (n=89) and CVID patients (n=36). Of the 12 individuals with total PCP results <9.8 mg/L, 11 (91.6%) carried a diagnosis of CVID. In contrast, of the 51 samples with total PCP results >270 mg/L, all were identified as healthy controls. Within the lower indeterminate range (9.7-40 mg/L), 11 out of the 12 (91.6%) were from patients with CVID; in the higher indeterminate range (180-270 mg/L), 11 out of the 12 (91.6%) were from healthy individuals. Lastly, the range of 40-180 mg/L showed the

most overlap, consisting of 13 (34.2%) CVID patients and 25 (65.8%) healthy controls. **Conclusion:** A specific algorithm for the screening of pneumococcal IgG serotype antibodies has been developed in which all referrals would be measured using the total PCP IgG ELISA as a screen and then reflexed to pneumococcal serotyping either automatically or based on physician request.

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Retrospective review of infliximab quantitation and anti-infliximab test results

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Background: Infliximab (IFX) is a monoclonal therapeutic antibody that inhibits tumor necrosis factor (TNF) and is used for the treatment of chronic inflammatory diseases, such as inflammatory bowel disease and rheumatoid arthritis. One of the factors associated with decreased concentrations of IFX and therefore a loss of response, is the formation of antibodies-to-infliximab (ATI). IFX and ATI quantitation are useful in helping to guide patient management decisions. **Objective:** The objective of this study was to assess correlations between age and gender with IFX concentration and ATI positivity rates for a population for whom this testing was ordered for routine clinical purposes. **Methods:** IFX results between April 7th, 2015 and May 26th, 2017 were extracted from the electronic medical record for 15,425 unique patients (male=7757, 50.3%; female=7668, 49.7%). Quantification of IFX was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on API 5000 triple quadrupole instrument (AB Sciex). Patient samples with IFX results ≤5.0 µg/mL were reflexed to ATI testing performed using a laboratory-developed electrochemiluminescent bridging immunoassay. Data and student t test were analyzed using Microsoft Excel. **Results:** Within this cohort of 15425 patients, 8641 patients (56%) had IFX results >5.0 µg/mL. Median (interquartile range, IQR) and mean±standard deviation (SD) IFX concentrations for males (n=4203, 48.6%; median age=22 years) were 11.0 µg/mL (11.4 µg/mL) and 16.4±14.4 µg/mL, respectively. Median (IQR) and mean±SD IFX concentrations for females (n=4438, 51.4%; median age=27 years) were 12.0 µg/mL (14.1 µg/mL) and 18.7±16.8 µg/mL, respectively. A total of 6784 patients (44%) had IFX results ≤5.0 µg/mL and were reflexed to ATI testing. Within the cohort of patients tested for ATIs, there was an equal distribution between males (n=3554, 52.4%) and females (n=3230, 47.6%). Of the patients tested for ATIs, 6,016 patients (88.7%) that had no detectable ATI (ATI<50 U/mL) and 768 patients (11.3%) were positive for ATI (ATI≥50 U/mL). Again, there was an equal distribution of patients who were positive for ATIs between males (n=390, 49.6%) and females (n=379, 49.3%). Within the male population, individuals ≤50 years of age had an ATI positivity rate of 9.9%, compared to individuals >50 years with a positivity rate of 15.8% (p≤0.01). However, median ATI concentrations for the male age groups ≤50 years and >50 years showed no significant differences at 229 U/mL and 298 U/mL, respectively (p=0.115). Similar trends were observed within the female population, with ATI positivity rates of 11.0% and 14.4% for age groups ≤50 years and >50 years, respectively (p≤0.05). Median ATI concentrations for the female age group ≤50 years was 195 U/mL and >50 years was 228 U/mL (p=0.686). **Conclusion:** There were no significant differences in median concentration of IFX or ATI positivity rates between males and females. However, a significant increase in the percentage of positive ATIs was found between individuals ≤50 and >50 years of age in both males and females. Whether this increase reflects changes in the immune system with age or a longer time period of IFX treatment remains to be determined.

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Vitamin D and disease activity in patients with rheumatoid arthritis

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Backgroud: Vitamin D has immunomodulatory effects in a wide variety of chronic diseases (autoimmune, cardiovascular, oncological, ...). Its deficiency has been related to the presence and activity of autoimmune diseases such as rheumatoid arthritis (RA). 25-hydroxyvitamin D or 25(OH)D has a half-life of 2-3 weeks, making it the best serological biomarker of vitamin D status in the body. The main objective of the study is to evaluate the relationship between 25(OH)D levels and the severity of the disease in patients with RA. The following objectives were established: 1) Compare the levels of 25(OH)D between healthy controls and patients with RA. 2) Compare the levels of 25(OH)D according to the disease activity in patients with RA. 3) Evaluate the status of vitamin D in patients with RA. 4) Evaluate the correlation between 25(OH)D levels and the clinical parameters of the disease: number of painful joints (NAD), number of inflamed joints (NAI), DAS28 activity index and biomarkers (PCR, FR and anti-CCP). **Material and methods:** Case-control study performed on 41 healthy controls (9-men:32-women, age=51 years (45-57)) and 78 patients with RA (18-men:60-women, age=57 years (49- 65)): 29 patients in remission and 49 patients with active disease. The activity of the disease was evaluated with the use of the DAS28 index: remission (DAS28 <2.6) and active disease (DAS28> 2.6). Vitamin D status was defined as deficiency (25(OH)D<20 ng/mL), insufficient (25(OH) D between 20-30 ng/mL) and adequate (25(OH)D between 30-100 ng/mL). The variables studied were 25(OH)D, DAS28, NAI, NAD, PCR, FR and anti-CCP. The Mann-Whitney test was used for comparisons between 2 quantitative variables, the Chi-square test for comparisons between qualitative variables based on contingency tables and the Spearman correlation analysis to study the correlation between variables. A p<0.05 was considered statistically significant. **Results:**
Objective 1: The levels of 25(OH)D were lower in patients with RA vs. healthy controls: 24.30 (18,30-31,40) ng/mL vs. 30.20 (24.80-43.30) ng/mL, respectively (p=0.002). **Objective 2:** In patients with RA; the levels of 25(OH)D were lower in patients with active disease vs. disease in remission: 21.20 (15.70-27.20) ng/mL vs. 31.40 (24.4-44.00) ng/mL, respectively (p<0.0001). **Objective 3:** 31% of patients with RA had vitamin D deficiency, 36% inadequate vitamin D and 33% adequate levels of vitamin D. According to clinical activity based on DAS28, adequate levels were associated with disease in remission and deficient levels were associated with clinical activity (p=0.04) (see table). **Objective 4:** An inverse correlation was obtained between the levels of 25(OH)D with the activity index DAS28 (r=-0.566, p<0.0001), NAD (r=-0.430, p<0.0001), NAI (r=-0.337, p=0.003). No significant correlation was observed with the biomarkers (anti-CCP, FR and PCR). **Conclusions:** The levels of 25 (OH) D are lower in patients with RA than in healthy controls. Vitamin D deficiency is associated with the clinical activity of the disease. The quantification of serum 25 (OH) D levels and, consequently, vitamin D supplementation should be considered in the management of patients with RA.

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Pro and Anti-inflammatory miRNA balance in chronic opiate abusers based on duration of addiction

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Background: Opium is the second most commonly abused substance after tobacco in developing countries of the Middle East region and in many Asian nations. Opioids can interfere with the immune system by participating in the function of the immune cells and causing modulation of innate and acquired immune responses. But there is dearth of data on immune regulatory miRNAs in opiate addicts. **Aim:** The current study aimed to analyse the balance of pro and anti-inflammatory miR 155 (pro-inflammatory) and 187 (anti-inflammatory) in chronic male opiate addicts based on the duration of dependence. **Methods:** The study included 46 chronic opiate dependents and 40 healthy control male subjects attending the de-addiction clinic at a non-government organization and a tertiary care centre. The study subjects were chronic opiate abusers (pure opium/ heroin) belonging to the age group 18 to 50 years. The subjects were analysed clinically for blood pressure. The venous blood samples were analysed for inflammatory cytokines IL-10, IL-6, TNF-alpha using ELISA and processed for extraction of miRNA. Kit based extraction of miR 187 and 155 was done (Qiagen) and c-DNA synthesized and stored at -80^{0}C. Relative gene expression of miR 187 and 155 was done by real time PCR and ΔCq values calculated using GAPDH as housekeeping gene. The data was analysed statistically by students' paired T test, Pearson's correlation and multiple regression analysis (after adjusting for dependency months as greater than 100 months and lesser than 100 months). **Results:** The study subjects had a mean age of 32±9.0 years (opiate dependents) and 33±7.34 years (controls). The diastolic blood pressure (DBP) was significantly reduced in the opiate dependent subjects (p=0.001). The serum levels of IL-10 and TNF-alpha were significantly raised in the opiate dependents as compared to controls (p=0002 and 0.03) whereas IL-6 was significantly reduced in opiate dependents (p=0.001). The ΔCqmiR155 (12.328±7.037, p=0.0311) and ΔCqmiR187 (15.242±2.361, p=0.0219) in opiate dependents were significantly higher than control subjects i.e. relative gene expression of miR155 is greater than miR187 in these patients. Multiple regression analysis carried out with the patients having >100 months dependence, showed that ΔCqmiR155 was significantly positively correlated with IL-6, TNF-alpha, SBP and negatively with IL-10. Further, ΔCqmiR155 was significantly predicted (F= 12.03, p=0.004,R^2=0.923) by IL-6, IL-10, TNF-alpha, SBP and memory loss. Patients with dependence ≤ 100 months ΔCqmiR155 was significantly positively associated with loss of memory. Multiple regression analysis adjusted for dura-

tion of dependence showed non-significant prediction by IL-6, IL-10, TNF-alpha. Similarly, ΔCqmiR187 in patients with duration >100months showed significant positive association with IL-10 but those with duration ≤ 100 months showed a non-significant association to any of the inflammatory cytokines. **Conclusion:** The study results suggest that opiate addiction causes an imbalance of immune miR 155 and 187. Further the duration of opiate addiction can prove to be crucial in the development of immune-modulation and needs further longitudinal studies.

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Comparison of a conventional and a nucleosome linker-based anti-dsDNA ELISA

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Background: Autoantibodies against double-stranded deoxyribonucleic acid (dsDNA) are found almost exclusively in systemic lupus erythematosus (SLE) and present a well-established criterion for diagnosis. Anti-dsDNA autoantibodies can be detected by different methods. In a conventional ELISA the target antigen dsDNA is linked to the solid phase by negatively charged molecules. As these are prone to cause unspecific reactions, the Euroimmun Anti-dsDNA-NcX ELISA uses purified mono-nucleosomes instead. This study was designed to compare the diagnostic performance of a conventional ELISA with the NcX-ELISA. **Methods:** Autoantibodies of class IgG against dsDNA were measured in serum samples from 204 SLE patients (including patients with inactive SLE under treatment) and 128 non-SLE patients (including rheumatoid or psoriatic arthritis, osteoarthritis, (fibro)myalgia, Raynaud's phenomenon, cutaneous sclerosis and vasculitis) using a conventional anti-dsDNA ELISA (QUANTA Lite®, Inova, USA) and the nucleosome linker-based Anti-dsDNA-NcX ELISA (Euroimmun, Germany). **Results:** The QUANTA Lite® dsDNA ELISA revealed a diagnostic sensitivity of 22.1 % at a specificity of 84.4 % and the anti-dsDNA-NcX ELISA a diagnostic sensitivity of 29.4 % at a specificity of 93.0 %. If the specificity of the QUANTA Lite® dsDNA ELISA is set to equivalent 93.0 %, ROC curve analysis demonstrates a sensitivity of only 15.2 %. **Conclusion:** The Anti-dsDNA-NcX ELISA, utilizing purified mono-nucleosomes as a linker for dsDNA, is superior in sensitivity and specificity than a conventional anti-dsDNA ELISA.

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Preferential kappa selection in circulating human immunoglobulins: an interesting conundrum for immunoglobulin gene rearrangement

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Background: The circulating antibody population is commonly used as a surrogate marker for B-cell gene selection. Analysis of the antibody repertoire by mass spectrometry showed unexpected bimodal mass distributions in total human serum Kappa (κ) light chain (LC) but not in lambda (λ) LCs (Barnidge et al. 2015). The secondary κ population (heavy-κ), identified through peak modeling and sequence analysis, had an average molecular mass of 24,250Da (about 750Da greater than the standard κ distribution). The presence of an unexpected heavy-κ population suggests that κ LC gene rearrangement or Ig class switching may be less random and more deterministic than previously thought, and therefore isotype/subclass specific. The objective of this study was to determine the reference ranges for a heavy-κ population in the normal adult antibody repertoire for Ig isotypes and IgG subclasses. **Methods:** Residual normal adult serum samples (N=162) were immunoenriched for IgA, IgG, IgG1, IgG3, IgG4, and IgM isotypes using heavy chain specific affinity resins (CaptureSelect ™, Thermo Scientific). Immunoglobulin isolates were further purified using an immunoaffinity resin specific to κ LC, to create final purifications of IgGκ, IgG1κ, IgG3κ, IgG4κ, IgMκ and IgAκ. Isolates were then reduced and spotted onto a 96-spot MSP polished steel target plate. MALDI-TOF MS spectra were acquired using a Bruker microflex LT, summing 500 laser pulses. Each resulting spectrum was analyzed using automated peak modeling as previously described (Mills et al. 2016) to calculate the area under each curve and the heavy-κ/ κ ratio of each Ig class and subclass. **Results:** Of the 162 samples tested, the numbers of qualified spectra used for each of the calculations were as follows: 94 IgAκ, 162 IgGκ, 126 IgMκ, 53 IgG1κ, 40 IgG3κ, and 53 IgG4κ. The average ratio of heavy-κ LC to normal κ LC ranged from 0.58 to 0.35. IgG1 had the highest proportion of heavy-κ LC (0.58) followed by IgM (0.540) then IgG3 (0.482), total IgG (0.479), and IgG4 (0.398). IgM contained a significantly greater heavy-κ/ κ ratio than IgG3 ($p=0.0367$), total IgG ($p=0.00265$), IgG4 ($p<0.0001$), and IgA ($p<0.0001$). **Conclusions:** A population of heavy-κ LCs, unique from the normal mass κ LC distribution was paired to all isotypes investigated (IgA, IgG, IgG1, IgG3, IgG4, and IgM). A significantly higher amount of heavy-κ LC was found in IgM as compared to other isotypes except IgG1, suggesting that circulating human immunoglobulins have a deterministic mechanism to select one type of κ LC based on Ig class and subclass. These findings present an interesting conundrum for current gene light chain selection theory.

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UFO-ANA: Cryptic antinuclear autoantibody target antigens revealed

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Background: Antinuclear autoantibodies (ANA) as defined by a pattern on HEp-2 in indirect immunofluorescence assay (IFA) are often not confirmed with a test using established nuclear autoantigens. We developed a strategic approach to identify such "Unknown Fluorescence Objects (UFO)". **Methods:** 36 serum samples with pre-determined UFO-ANA against unknown nuclear autoantigens as determined by IFA and immunoblot were collected in a Clinical Immunological Laboratory in Lübeck, Germany. UFO-ANA sera and controls were incubated with different substrates, including HEp-2 cells and monkey liver cryosections fixed on cover glass, homogenate of harvested HEp-2 cells, cell-free supernatant and nuclei to form immune complexes. These were subsequently extracted with detergents, enriched with protein G magnetic beads, and subjected to SDS-PAGE and Western blot with the autologous index serum as the immunoprobe. Immunoreactive proteins were identified by MALDI-TOF mass spectrometry. The candidate autoantigens were recombinantly expressed in *E.coli* and HEK293. The recombinant proteins were incubated with the corresponding index serum and controls in Western blot to verify the identification. Moreover, they were tested for their ability to neutralize the respective UFO-ANA reactivity in IFA. **Results:** IP using fixed HEp-2 cells or cell fractions was able to isolate autoantigens that were detected by the autologous sera in Western blot. Among them, IP using fixed cells resulted in lowest number of irrelevant proteins in the precipitate. IP with nuclear extract and monkey liver did generally not lead to conclusive identifications. Five target autoantigens were identified by mass spectrometry: DNA-directed RNA polymerase II subunit RPB1 (POLR2A) with three UNA sera and tight junction protein zonula occludens-1 (TJP1), ATP-dependent RNA helicase A (DHX9), proteasome activator complex subunit 3 (PSME3) and structural maintenance of chromosomes flexible hinge domain-containing protein 1 (SMCHD1) with individual other UNA sera. All candidate proteins could be recombinantly expressed and prepared in sufficient amounts for subsequent immunoassays. The recombinant proteins reacted with the corresponding index serum in Western blot but not with >80 control sera. Moreover, all recombinant proteins were able to abolish the UFO-ANA reaction of the respective index serum in IFA. **Conclusion:** Five candidate nuclear autoantigens were identified, among them two candidates that have not been described before. They were successfully verified by demonstrating specific reactivity of their recombinant homologues with the corresponding index serum. The strategy is currently being applied to further UFO-ANA. The prevalences of autoantibodies against these antigens can then be determined in large scale in cohorts of patients with systemic autoimmune diseases.

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Quantitating the M-protein: A comparison between the perpendicular drop and the tangent skimming methods on Agarose Gel (AGE) and Capillary Zone (CZE) Electrophoresis

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BACKGROUND: Serum protein electrophoresis (SPEP) is a staple test for detecting and measuring the M-protein. Different techniques exist for quantitating M-proteins: perpendicular drop (PD) or tangent skimming (TS). It's been reported that TS would be more accurate at lower concentrations (<1g/dL) and favored for quantitation. This study compared PD and TS methods in different institutions using CZE or AGE on gamma-migrating M-proteins. **METHODS**: Residual waste serum with physician-ordered SPEP was used to create hypogamma, normal, and hypergamma background samples, then divided into 2 groups (n=6). Each pool was spiked with elotuzumab (Elo) or daratumumab (Dara) monoclonal antibodies (mAbs). MAb concentration in each pool ranged from 1-0.02g/dL. SPEP was performed in duplicate in different analytical runs (n=62). Mayo Clinic (MC) used a SPIFE SPE Vis agarose gel using a Helena SPIFE 3000 unit and Helena QuickScan 2000 densitometer, gating mAbs first with PD, then with TS. University of Michigan-Ann Arbor (UM), performed SPEP by CZE on the Sebia Capillarys 2, and mAb concentrations were determined using TS. A recovery within 80-120% from the expected concentration was considered acceptable. **RESULTS**: Percent recovery was calculated for three sets of data gated by different methods (Table). Zero percent (0/66) of samples analyzed by AGE/TS fell within the acceptance criteria (80-120%). Thirty percent (20/66) of all samples analyzed by AGE/PD and 59% (39/66) of samples analyzed by CZE/TS met those criteria. Lowest measured concentrations with acceptable recovery were 0.02g/dL for CZE/TS and 0.2g/dL for AGE/PD. The hypergamma background affected the PD method more significantly than TS. **CONCLUSION**: Our findings suggest that SPEP performed by CZE/TS showed improved accuracy compared to AGE/PD or AGE/TS at mAb concentrations <1g/dL, and that the system used for quantitation also plays a critical role in the accuracy of the reported result.

Expected [], g dL		1 (0.8-1.2)	0.8 (0.64-0.96)	0.5 (0.4-0.6)	0.3 (0.24-0.36)	0.2 (0.16-0.24)	0.1 (0.08-0.12)	0.05 (0.04-0.06)	0.02 (0.016-0.024)
Dara-Hypo	UM TS	0.83	0.7	0.44	0.26	0.17	0.08	0.04	0.01
	MC TS/PD	**0.75**/0.88	**0.58**/0.71	**0.33**/0.48	**0.19**/0.33	**0.11**/0.24	*	*	*
Dara-Normal	UM TS	0.83	0.69	0.4	0.25	**0.15**	**0.06**	0.04	*
	MC TS/PD	**0.78**/0.97	**0.58**/0.79	**0.33**/0.54	**0.19**/0.37	**0.11**/0.29	*	*	*
Dara-Hyper	UM TS	0.82	0.73	**0.37**	0.25	**0.13**	**0.07**	**0.07**	NA
	MC TS/PD	**0.75**/1.12	**0.62/1.07**	**0.33/0.84**	**0.18/0.63**	*	*	*	NA
Elo-Hypo	UM TS	0.8	0.65	0.4	0.24	0.16	0.08	0.04	0.02
	MC TS/PD	**0.77**/0.98	**0.60**/0.81	**0.29**/0.49	**0.17**/0.34	**0.12/0.26**	*	*	*
Elo-Normal	UM TS	**0.77**	**0.61**	0.4	0.24	0.17	0.08	0.04	0.02
	MC TS/PD	**0.76**/1.18	**0.54**/0.95	**0.3/0.66**	**0.17/0.48**	**0.1/0.39**	**0.03/0.18**	*	*
Elo-Hyper	UM TS	**0.79**	**0.57**	0.4	0.24	0.17	**0.07**	**0.03**	NA
	MC TS/PD	**0.69/1.45**	**0.56/1.39**	**0.32/1.06**	**0.19/0.83**	**0.12/0.79**	*	*	NA

(*) M-protein not quantified, (NA) dilution not tested

A-391

Discrepant CMV IgM Immunoassays: A Comparison between Bio-Rad RCM and TORCM with Poor Positive Agreement

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Background: Cytomegalovirus (CMV) infection in the general, immunocompetent, population is mostly asymptomatic. However, CMV infection is of great concern in patients with weakened immune systems; and therefore, determination of CMV infection status in immunocompromised patients is of utmost importance, including patients with HIV infection, patients receiving treatment for autoimmune conditions, infants with not yet fully developed immune systems, and organ transplant recipients, as well as an important component of pre-transplant testing. CMV tends to infect transplant organs and is thought to contribute to chronic rejection. CMV IgM immunoassays aid in establishment of primary CMV infection, as well as secondary infection, with a different strain or reactivation of a latent CMV strain. **Methods:** In this study we evaluated two semi-quantitative CMV IgM immunoassays from Bio-Rad®: the latest generation TORCM and the previous generation RCM. Positive and negative quality controls were run in duplicates for 10 days to assess precision of the TORCM assay. A total of 40 patient serum samples were tested by the two different assays on the same instrument platform (Bioplex 2200, Bio-Rad). The discrepant results were compared to a third commercially available immunoassay (DiaSorin LIAISON XL) and were also correlated with available CMV PCR test data for each patient, if tested at the same time period. **Results:** The new TORCM assay shown good precision with 100% agreement for both positive and negative control. Of the 40 tested samples, a total of 21 samples were tested negative by both TORCM and RCM, with 100% negative agreement between the two assays. Out of 18 samples tested positive by the RCM assay, 7 samples had discrepant results by TORCM assay, with only 61% positive agreement between the two assays. One remaining sample tested equivocal on RCM and positive on TORCM. Of the 7 discrepant results, 6 agreed between TORCM and DiaSorin. CMV PCR test data was available in 9 of the 18 positive samples by RCM assay. When compared to the available CMV PCR data, both assays shown a poor total agreement with the PCR results: RCM, 33%; TORCM, 44%. **Conclusion:** This study demonstrates poor positive agreement between the two Bio-Rad CMV IgM immunoassays. These results warrant further test comparison with standard molecular data, and further demonstrate a concerning and inherent lack of positive agreement between two different CMV IgM immunoassays with significant potential impact in patient care.

Wednesday, August 1, 2018

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

Animal Clinical Chemistry

B-001

Tube Type and Centrifugation Conditions Contribute to Hemolyzed Serum Samples in Rats

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Objective: An investigation was performed to determine how tube type and centrifugation conditions contribute to hemolysis in clinical chemistry samples from rats. Blood from 10 (5 male / 5 female) Sprague Dawley (SD) rats was collected and aliquoted into K_2EDTA and multiple different serum collection tubes (BD367977, BD367814, and Capiject T-MG). Blood from the serum collection tubes was processed to serum using one of two different centrifugation protocols (3500 RPM for 15 minutes or 2700 RPM for 10 minutes) to assess how tube volume, inclusion of serum separator gel, and centrifugation affected hemolysis. **Methodology:** K_2EDTA blood was used to assess routine hematology parameters (Advia® 2120 hematology analyzer), osmotic red blood cell (RBC) fragility, and RBC fragility using a mechanical hemolysis model (rapid repeated passage through a needle). Serum aliquots from each tube were evaluated for clinical chemistry parameters and hemolytic index (Abbott ARCHITECT c16000 clinical chemistry analyzer). Clinical chemistry and hemolytic index data were compared to the laboratory's current centrifugation conditions and tube size/type (designated control). **Results:** Compared to females, male RBCs had more hemolysis after exposure to the mechanical fragility model. Compared to control, serum from small volume tubes and serum from tubes centrifuged at lower speed and shorter duration had lower mean hemolytic index values, decreased lactate dehydrogenase activities, and aspartate aminotransferase activities. The absence of a serum separator gel had no effect on hemolytic index or enzyme activity. **Conclusions:** Serum tube selection and centrifugation conditions can contribute to hemolysis in SD rat serum samples. Smaller tubes and slower centrifugation speeds resulted in less hemolysis. Tube selection and centrifugation conditions provide opportunities to control hemolysis, an important contributor to pre-analytical variability, in SD rat serum samples.

B-002

CD4 Count and Biochemical Parameters in HIV Positive Individuals of Western Nepal

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Background: The human immunodeficiency virus is one of the most prime rising infections with multiple impacts on persons, families, communities, society and the entire country. The number of HIV infection is increasing every year in Nepal and estimated numbers of people with HIV were 32735, male (20232) and female (12503) in 2016. Objectives: To see the status of CD4 count and biochemical parameters in HIV positive individuals and correlation of CD4 with liver enzymes. **Materials and method:** Data were collected from 146 HIV seropositive individuals at ART center, Western regional Hospital, Pokhara, Nepal. The blood samples were collected and analysed for CD4 count and biochemical parameters at Manipal Teaching Hospital, Pokhara, Nepal. The data were analysed using SPSS 16. **Result:** The mean±SD age of HIV infected subjects were 37.74±13.28 and female (55.6%) were infected more in compare to male (44.4%). The mean±SD of biochemical parameters were RBS (93.14±15.84), Urea (22.05±6.00), Creatinine (0.95±0.20), Total Protein (7.78±0.80), Albumin (4.65±0.51), AST (22.56±10.59), ALT (28.75±12.24), ALP (85.49±23.28), Uric acid (5.41±1.46), Magnesium (1.58±0.55), Amylase (48.52±11.3), Lipase (37.27±12.01), CK-MB (24.74±17.08) and CD4 count (457.27±238.26). The most of the cases were from Kaski (59.87 %) followed by Tanahu (13.58 %), Parabat (10.49 %) and Syngja (5.55 %) and least from Lamjung (1.85 %) Gorkha (1.23 %) districts. The most of the cases were from 30-39 and 40-49 years age group followed by 50-59 years age group. The cases were also found in <20 years age group. In pearson correlation, the CD4 count were negatively correlated with AST (-0.012), ALT (-0.061) and ALP (0.089). **Conclusion:** The biochemical parameters were normal in HIV infected individuals and CD4 count showed negative correlation with liver enzymes.

B-003

Olive oil diet and supplementation with omega 3

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The thymus shows important functional changes in response to nutritional disorders. The aim of this work was to analyze the effect of diet containing olive oil, with and without the supplementation with omega 3, on serum and thymus' fatty acid profiles of growing rats. Weanling Wistar rats were fed during 10 days with normocaloric dietary and fat was provided by olive oil (O group). The other group received the same diet supplemented with 24mg/day of fish oil (OS group). Control group(C) received normocaloric diet (AIN´93). Serum and thymus fatty acids profiles were determined by gas chromatography. Statistical analysis used ANOVA and Dunnett as post test. This work was approved by the ethics committee of the University of Buenos Aires and in conformance with the FASEB Statement of Principles for the use of Animals in Research and Education. Results of oleic, linoleic, alfa-linolenic (ALA), EPA and DHA acids expressed as %Area were: SERUM: OLEIC O: 23.44±3,68^{c}; OS: 18,31±2,22^{b}; C: 10,60±2,01^{a}. LINOLEIC O: 12,44±1,65^{b}; OS: 12,98±4,31^{b}; C: 18,27±2,81^{a}; ALA O: 0,30±0.09^{b}; OS: 0,32±0,08^{b}; C: 0,92±0.34^{a}; EPA O: 0.65±0.17^{a}; OS: 1.63±0.49^{b}; C: 0.80±0.23^{a}; DHA: O: 1,57±0,58^{a}; OS: 4,00±1,70^{b}; C: 1.33±0.19^{a}. THYMUS: OLEIC O: 21,54±5,92; OS: 24,40±5,04; C: 18,22±3,23. LINOLEIC O: 5,90±0,56^{b}; OS: 6,5±0,61^{b}; C: 10,89±2,18^{a}; ALA O:0,27±0.02^{b}; OS: 0,30±0,07^{b}; C: 0,49±0,19^{a}; EPA O: 0,49±0,28; OS: 0,50±0,13 ; C: 0,50±0,12; DHA: O: 0,47±0,10^{a} ; OS: 0.70±0,12^{b} ; C: 0,52±0,16^{a}. Media that did not present a letter (^{a,b}) in common, were different ($p<0.05$). In sera, O and OS groups showed lower ALA and linoleic acids levels and higher oleic acid levels, compared to C. The results suggest that the olive oil exacerbated omega 9 family with diminution of essential fatty acids. OS group presented high levels of EPA and DHA. In thymus, O and OS groups showed lower levels of ALA and linoleic acids than C. OS group only increased DHA. Fish oil supplementation increased DHA levels on serum and thymus, not modifying essential fatty acid low levels. EPA increase only in serum. The results suggest that dietary lipids provoked changes in serum and thymus fatty acids profiles. This work was supported by UBACyT : 20020150100011BA.

B-004

Haematological profile in capuchin monkey females *Sapajus libidinosus* according to ovarian cycle

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The aim of this study was verify difference on haematological profile and reproductive hormones in capuchin monkey females according to ovarian cycle. One premise required to maintain an animal species as a biological model is the comprehension and respect for the animal's ethology and the control on its reproduction in captive conditions. This knowledge allows for planning and implementation of an assisted reproduction program aiming to maintain a self-suficient colony and to avoid the capture of specimens from nature. Thus, this work aims to verify difference on haematological profile and reproductive hormones in capuchin monkey females according to ovarian cycle. Six tufted capuchin (*Sapajus libidinosus*) were used as subjects. They were adult females between 5 and 7 years old with 2.508±0.230 kg. All subjects presented a normal menstrual cycle. Trial lasted two continuous menstrual cycles (46 days). Animals were captured, and restrained by the handler for sampling of vaginal epithelial cells (daily) and peripheral blood (weekly). Blood was used to hemogram and leukogram. Blood serum was obtained by centrifugation at 2000 rpm for 5 min. Then, serum was used to measure the concentration of progesterone (P_4) and estradiol (E_2) in duplicates by radioimmunoassay (RIA). Data were analyzed using Kruskal-Wallis test to confirm differences between cellular population over the ovarian cycle. Haematological data were analysed using PROC MIXED and repeated measures. Morphological analysis of vaginal smears demonstrated three different menstrual phases (Table 1). There was no difference between ovarian phases for none of hametaological traits measured. No difference was observed in estrogen (E_2) concentration between ovarian phases and there was difference only between follicular and periovulatory phases for P_4. Capuchin monkey females do not present haematological profile difference between ovarian phases. Although the E_2 peak is recongized as a good indicator of ovulation,

in the present experiment, the increase on P_4 concentration was related with periovulatory period.

Tabela 1. Celular type proportion (%) according to ovarian cycle in capuchin monkey (*Sapajus libidinosus*).

Celular Type	Ovarian Phase			*P*
	Follicular	Periovulatory	Luteal	
Basal	$3,51^{Aa}$	$2,08^{Ba}$	$1,86^{Ba}$	0,0069
Parabasal	$45,67^{Ab}$	$13,63^{Bb}$	$13,37^{Bb}$	<0,0001
Intermediate	$41,67^{Ab}$	$66,97^{Bc}$	$69,00^{Bc}$	<0,0001
Superficial	$9,12^{Ac}$	$17,30^{Bd}$	$15,76^{Bb}$	<0,0001

Different upper case letters (A,B) in the same row and different lower case letters (a,b,c,d,) in the same column are significantly different (*P*<0.05) using the Tukey test.

B-005

Preclinical Investigations of Platelet Function using the Chrono-Log Model 700 Aggregometer

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The Chrono-Log Model 700 Aggregometer has been used to evaluate in vivo effects on platelet function in humans and preclinical species. We describe two in vitro investigations where the Chrono-Log Aggregometer was used to help characterize suspected platelet dysfunction observed during preclinical in vivo studies. The first investigation was to determine if in vitro exposure to Compound A in monkey or human platelet-rich plasma (PRP) could produce inhibitory effects on platelet function. Blood (anticoagulated with sodium citrate) was collected from 4 monkey and 5 human donors. PRP and platelet poor plasma (PPP) were prepared by centrifugation. Platelet counts for PRP were performed and adjusted to a count of ~250,000/µL with respective PPP for all samples. Compound A was diluted in 0.9% sodium chloride and added to PRP samples at a constant volume (25 µL) to achieve final drug concentrations ranging from 0.3 µM to 5 µM. Verapamil was prepared as a stock solution and diluted to obtain a final concentration of 0.175 mg/mL (356 µM) in PRP for use as a positive control. Platelet aggregation for each sample was measured on the Chrono-Log 700 using collagen as agonist. Results demonstrated a dose -dependent inhibition of collagen-induced platelet aggregation (%) with Compound A at concentrations of ≥1 µM (monkeys) and 5.0 µM (humans) when incubated with monkey and human PRP. These in vitro findings correlated with macroscopic observations of hemorrhage and petechiae in an in vivo monkey toxicology study at comparable systemic exposure and informed on potential translatability to humans. In contrast, Compound B had a potential platelet activation liability based on decreased platelet counts observed in an in vivo monkey study. We evaluated the ability of Compound B to elicit platelet activation in vitro using monkey PRP. Blood was collected from 4 stock monkeys, and PRP and PPP were prepared as described previously. Compound B (10 µL) was added to PRP samples to achieve final concentrations ranging from 0.001 µM to 0.5 µM and platelet aggregation (%) was measured for 10 minutes. Collagen (10 µL) was added to one tube of PRP from each monkey and served as a control for normal platelet aggregation. Addition of Compound B alone to monkey PRP caused 30% and 70% aggregation at concentrations of 0.1 µM and 0.5 µM, respectively. These findings provided pharmacokinetic thresholds for the desired pharmacologic effect relative to the platelet activation liability. The Chrono-Log 700 Aggregometer has proven to be a valuable tool for characterizing effects on platelet function in preclinical pharmaceutical development.

B-006

Effects of Erythropoiesis Stimulation on the Biomarkers of Endothelial Injury and Atherosclerosis Development in Apo E Knockout Mice

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Background: Atherosclerosis is a disease of large and medium-sized arteries, resulting from interactions between genetic and environmental factors, characterized by endothelial dysfunction, vascular inflammation, and build-up of lipids, cholesterol and cellular debris within the intima of the vessel wall. Atherosclerotic lesions develop as a result of inflammatory stimuli, subsequent release of various cytokines, proliferation of smooth muscle cells, synthesis of connective tissue matrix, and accumulation of macrophages and lipids. The ApoE-/- mice are the leading mammalian model organisms studied for accelerated atherosclerosis and for the dşscovery of mechanisms involved in atherosclerosis. Low-dose treatment with Aranesp may be a potential therapeutic tool to prevent endothelial injury and atherosclerosis development. In this study, we investigated the effects of Aranesp treatment during atherosclerosis progression and development in ApoE-/- mice fed with a standard diet compared to the wild-type C57BL/6 mice having the same genetic background as the control group. Our aim was to reveal the potential differences in various biochemical parameters on oxidative stress, inflammation and endothelial injury in ApoE-/- and control mice groups and to understand the effect of Aranesp on the studied parameters. **Material and Methods**: In order to study the effect of Aranesp on endothelial injury and atherosclerosis, we used apolipoprotein E-deficient (ApoE-/-) mice as the atherosclerotic mice model. We monitored atherosclerosis and plaque formation histochemically in ApoE-knock-out mice at early and late stages of atherosclerosis. ApoE-/- mice were splitted into 4 groups (10 animals each) which were injected Aranesp intraperitoneally at a dose of 0.1 µg/kg or saline for a period of 8 or 20 weeks (initial and advanced stages of atherosclerosis respectively). The results of two ApoE-/- mice groups injected Aranesp (early and late stages of atherosclerosis) were compared with the results of the corresponding saline injected ApoE-/- mice groups and the control (C57BL/6) mice. Lipid profile (total cholesterol, triglyceride), inflammation (CRP, IL-6, histamine), endothelial injury (ICAM-1, selectin) and oxidative stress markers (lipid peroxidation, protein oxidation) were measured in mice in different groups. **Results:** Lipid profile (total cholesterol, triglyceride), inflammation (CRP, IL-6, histamine), endothelial injury (ICAM-1, selectin) and oxidative stress markers (lipid peroxidation, protein oxidation) were significantly increased in four atherosclerotic groups compared to the control. Short-term Aranesp had no marked effects on serum lipid profile, or markers of inflammation and endothelial injury in ApoE-/- mice groups compared to the ApoE-/- mice not treated with Aranesp, but Aranesp significantly decreased 8-isoprostane and protein carbonyl content. Long term Aranesp treatment reduced oxidative stress in ApoE-/- mice significantly. **Conclusions**: This study contributes to the understanding and elucidation of the biochemical changes occurring during early and late stages of atherosclerosis development and effects of Aranesp regarding endothelial injury, inflammation, lipid profile, and oxidative stress markers.

B-007

Evaluation of Two Methods for Measurement of NT proANP in a Mouse Model of Heart Failure.

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Atrial natriuretic peptide (ANP) is a cardiac prohormone known to be released in response to cardiac muscle wall stretch. When secreted, ANP cleaves into the active peptide and a more stable inactive fragment, N-terminal proatrial natriuretic peptide (NT-proANP) that is routinely used in clinical medicine and also as a potential translational biomarker for drug or surgically-induced cardiac hypertrophy in preclinical safety studies. The objective of this evaluation was to compare the use of two commercially available assays for NT-proANP in a mouse model of heart failure. Transverse aortic constriction (TAC) in the mouse is a commonly used experimental model of heart failure that induces initial compensatory cardiac hypertrophy that transitions to heart failure and is considered a clinically translational animal model to evaluate the effects of new drug candidates on cardiac hypertrophy. Briefly, NT-proANP was measured in plasma samples from experimental control (sham) and TAC mice using the Meso Scale Discovery™ (Rockville, Maryland) rat NT-proANP kit (MSD) and the human-based EIA Biomedica™ (Vienna, Austria) proANP (1-98). All procedures were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline and were performed according to the guidelines of the Animal Welfare Act. The Biomedica assay is the validated method that has been used routinely to monitor for effects on NT-proANP in the TAC model. The MSD method was validated for use in rat GLP safety studies but had not been qualified for use with mouse plasma. Prior to use of the MSD method for the TAC model samples, we confirmed that the assay demonstrated acceptable precision (CV≤20%), dilutional linearity (R^2= 0.99) and long-term stability (2 months at -80°C). NT-proANP results for TAC mouse samples for both methods demonstrated comparable (4-fold) increases relative to sham control samples. These NT-proANP increases in TAC mice correlated with increases in gross whole heart and left ventricle weight normalized to body weight (63% and 72%, respectively). Although both NT-proANP methods were effective in discriminating heart weight changes in the TAC model, the MSD method has some advantages including robust electrochemiluminescence technology, broad dynamic range and improved cross-reactivity with rodent samples thus making this method a potentially better choice for use in assessing effects on this translational biomarker in rodent models of cardiac hypertrophy.

B-008

Pharmacological Enzymatic Action of NAD(P)H Quinone Oxidoreductase 1 Ameliorates Hepatic Metabolic Damage with Moderate Fibrosis Caused by Fasting Refeeding HFD

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Background: Because of unhealthy lifestyles, a large number of people are suffering from hepatic lipid accumulation and nonalcoholic steatohepatitis. Fasting-Refeeding with high fat diet (F-R HFD) promotes the development of hepatic steatosis and dysfunction in mice, but the effect in human is still unknown. NADH-quinone oxidoreductase 1 (NQO1) modulates intracellular NAD+/NADH ratio which plays a crucial role in cellular energy metabolism, and a dysregulated NAD+/NADH ratio is implicated in metabolic syndrome. We hypothesized that the pharmacological enzymatic action of NQO1 provides therapeutic effects in F-R mouse model of hepatic metabolic dysfunction with fibrosis. **Methods:** In this study, we designed to understand the fasting and refeeding processing to a meal, adult C57/BL/6J male mice were fed either a normal diet (ND: 12% of total calories from fat) or randomly fasted for 24 h and refed high fat diet (HFD: 60% of total calories from fat) for 24 h, and orally administrated with β-lapachone (βL), a well-known natural substrate of NADH:quinone oxidoreductase 1 (NQO1) for 12 weeks. Markers of oxidative stress and apoptosis as well as mediators of hepatic fatty acid metabolism were assessed by histopathological, Western-blot, real-time PCR and biochemical assays. **Results:** Here, we showed that 24 h refeeding with HFD after 24 h fasting in mice for 12 weeks results hepatic damage as compared to ND-fed mice assessed by hepatic morphology and cell death, and hepatic biomarkers. Our detailed analysis revealed that hepatic lipid formation is enhanced, and hepatic levels of free fatty acid, triglyceride and cholesterol are increased by F-R HFD. In addition, NF-kB is activated and consequently induces the proinflammatory mediators and intercellular ROS levels along with fibrotic markers in liver tissue. However, daily oral administrations of βL attenuate hepatic steatosis as well as the expression of srebp-1c, acetyl-CoA carboxylase (ACC) and hepatic lipid metabolism, and systemic inflammation and oxidative stress in liver by reduction of the acetylating of NFkB-p65 and the mRNA levels of proinflammatory cytokines caused by F-R HFD. Furthermore, we confirmed that βL increases the cellular NAD+ levels by NQO1 enzymatic action, prevents hepatotoxic effects during F-R HFD in liver through the regulation of PARP-1 and SIRT1 activity. **Conclusion:** This study is the first to demonstrate that enzymatic action of NQO1 has a hepatoprotective effect that is mediated by F-R with HFD via modulation of cellular NAD+/NADH ratio. Herein, our results provide strong evidence that βL could be a new therapeutic target for the prevention of F/R HFD-induced hepatic metabolic damage with moderate fibrosis.

B-009

Performance of Sysmex XN-1000V™ Automated Hematology Analyzer Compared to the Siemens ADVIA ® 120 in Mice and Rats, Part I: *Comparison of automated complete blood counts and reticulocyte parameters.*

J. M. Schroeder, D. M. Hamlin, A. E. Schultze. *Eli Lilly and Co., Indianapolis, IN*

BACKGROUND: Side-by-side studies comparing the recently released Sysmex XN-1000V™ to the Siemens ADVIA® 120 will provide insight into performance and capabilities for use in toxicological and efficacy studies for drug discovery or nonclinical development studies. The Sysmex XN-1000V, an automated veterinary hematology analyzer, utilizes laser light, impedance, fluorescent stains and flow-cytometry to analyze whole blood determining CBC, reticulocyte, and WBC counts. **OBJECTIVE:** The purpose of this study was to evaluate the Sysmex XN-1000V for determining complete blood counts (CBC), red cell indices, and reticulocyte counts in whole blood from mice and rats compared to the Siemens ADVIA 120. **METHODS:** Whole blood samples were collected from healthy untreated CD-1 mice and Sprague-Dawley rats from two distinct cohorts. Group 1 consisted of 40 rats and 85 mice. Group 2 was collected approximately 6 months later and consisted of 20 rats and 30 mice. Blood samples were analyzed within 4 hours of collection using both the Sysmex XN-1000V and the Siemens Advia 120 automated hematology analyzers with multi-species software. Analysis included CBC, total WBC and reticulocyte counts. Data from each collection were analyzed separately and then combined for further analysis to increase the N and assess variation between analysis events. Both methods for platelet analysis on the Sysmex XN-1000V (impedance for Group 1 and fluorescence for Group 2) were tested in comparison to the Advia 120 light scattering mode. Method correlation data from all samples were determined using EP Evaluator (Data Innovations LLC) for regression statistics including random error (Standard Error of the Estimate - SEE) and systematic error (absolute and percent bias). **RESULTS:** Measured parameters from individual cohort analyses (WBC, RBC, HGB, MCV, absolute and relative reticulocyte counts) showed very good to excellent correlation for both species (R≥0.91) with the majority of these parameters displaying excellent correlation (R>0.95). Hematocrit showed good correlation for both species (R≥0.89). While mice showed excellent consistency and correlation for reticulocyte counts (R=0.96-0.97), MCH and MCHC showed a little more variability within acceptable ranges. Rats showed significant variability between groups for these parameters. Platelets correlated consistently for both species with mouse R=0.90 or 0.92 and rats R=0.78 or 0.79. Upon combined data analysis from the two cohorts, N was increased to 60 rats and 115 mice. Measured parameters retained high correlation values for both species (R≥0.91). Reticulocyte counts in mice also showed excellent correlation (R=0.96). Both species maintained acceptable correlation on the combined data for HCT, MCHC, MCH and PLT (R≥0.71). Bias overall was ≤ 10.9% with the exception of platelets in mice (22.6%) and reticulocyte counts (32.4% in rats and R=17% in mice). **CONCLUSIONS:** The Sysmex XN-1000V showed overall consistent, comparable performance to the Siemens Advia 120 (low bias and acceptable to very good correlation) for CBC and reticulocyte analysis for laboratory mice and rats. While there were some differences between groups of animals collected at different time periods, the correlation values for all parameters overall were in the acceptable to excellent range indicating that the Sysmex XN-1000V is a reliable platform for hematology analysis in rodents.

B-010

Analysis of serum HDL subclass from rats with non-alcoholic fatty liver disease induced by high-fat and high-cholesterol diet

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Background: Non-alcoholic fatty liver (NAFL) associates with obesity, insulin resistance, hypertension, and dyslipidemia, and can progress to non-alcoholic steatohepatitis (NASH) characterized by hepatocyte injury, inflammation and fibrosis. However, mechanisms and risk factors involved in the progression to NASH remain poorly understood. Dietary cholesterol is well known to induce NAFL/NASH in animal models, and increase HDL-cholesterol (HDL-C) levels, especially along with apoE-rich HDL subclass. However, it is unknown whether this HDL subclass is involved in the development of NASH. Therefore, we compared HDL subclass between dietary-induced rat models of NAFL or NASH. **Methods:** Wister Kyoto (WKY) and SHRSP5/Dmcr rats were divided into four groups (n=2-5/group), and fed with stroke-prone (SP: 20.8 % crude protein, 4.8 % crude lipid, 3.2 % crude fiber, 5.0 % crude ash, 8.0 % moisture, and 58.2 % carbohydrate) or high-fat and high-cholesterol (HFC: a mixture of 68 % SP diet, 25 % palm oil, 5.0 % cholesterol, and 2.0 % cholic acid) diets. NAFL and NASH were induced in WKY and SHRSP5/Dmcr rats by the HFC diet, respectively. After eight weeks of HFC or SP diet feeding, serum HDL subclass was evaluated. Total HDL factions were isolated from whole sera by the polyethylene glycol precipitation method, and applied into a cation-exchange column (HiTrap SPHP, GE healthcare) for separating apoE-rich HDL. **Results:** On SP diet, total HDL-C and apoE-rich HDL-C levels were lower in SHRSP5/Dmcr (48.1 mg/dL and 27.5 mg/dL, respectively) than WKY rats (125.9 ± 12.7 mg/dL and 89.7 ± 7.9 mg/dL, respectively). The HFC diet induced a significant increase in apoE-rich HDL-C and decrease in apoE-poor HDL-C in both rats. ApoE-rich HDL-C levels were significantly lower in SHRSP5/Dmcr with NASH compared to WKY with NAFL (58.6 ± 12.9 mg/dL vs. 107.6 ± 8.8 mg/dL), and apoE-poor HDL-C levels were also lower in SHRSP5/Dmcr with NASH (10.2 ± 2.5 mg/dL vs. 24.1 ± 8.4 mg/dL). Furthermore, SHRSP5/Dmcr with NASH had significantly higher ratios (%) of free cholesterol to total cholesterol (FC/TC ratio) both in apoE-rich and apoE-poor HDL subclasses (19.7 ± 5.1 % and 11.5 ± 1.9 %, respectively), compared to WKY with NAFL (11.7 ± 2.3 % and 6.0 ± 1.5 %, respectively). There was no significant difference in non-HDL-C levels between the rats with NASH or NAFL. **Conclusion:** The present observations suggest that apoE-rich HDL may protect against free cholesterol accumulation in the liver, and the higher FC/TC ratio in HDL as well as the lower level of HDL may be an important risk factor for the progression to NASH in diet-induced rat model.

B-011

Performance of Sysmex XN-1000V™ Automated Hematology Analyzer Compared to the Siemens ADVIA ® 120 in Mice and Rats, Part II: *Comparison of automated leukocyte differential counts and manual microscopic methods.*

J. M. Schroeder, D. M. Hamlin, A. E. Schultze. *Eli Lilly and Co., Indianapolis, IN*

BACKGROUND: The Sysmex XN-V Series™ of automated hematology analyzers was recently introduced with software for use in research animals. Studies comparing the Sysmex XN-1000V™ to the Siemens ADVIA® 120 and manual microscopic methods will provide insight into performance and capabilities for use in nonclinical drug development. The Sysmex XN-1000V is a flow cytometry-based whole blood analyzer that produces complete blood, reticulocyte, and WBC differential counts. A 5-part leukocyte differential count is produced by fluorescent staining of WBCs and laser-based measurements of Side Fluorescent Light (SFL), Side Scatter Light (SSL), and Forward Scatter (FSC). Mononuclear cells are further separated by Sysmex Adaptive Flagging Algorithm for Shape-recognition (SAFLAS) technology. **OBJECTIVE:** The purpose of this study was to evaluate the Sysmex XN-1000V for determining differential leukocyte counts in blood from mice and rats compared to the Siemens ADVIA® 120 and manual microscopic reference methods. **METHODS:** Blood samples from healthy untreated animals (40 Sprague-Dawley rats and 85 CD-1 mice) were analyzed with the Sysmex XN-1000V and the Siemens ADVIA 120 using multi-species software. Manual differential counts were performed by two technologists counting 200 leukocytes each on separate Wright-stained blood smears for each animal. Manual differential values were averaged for comparison to instrument counts. Each instrument was compared to manual differential counts using percent values. Automated differential counts were evaluated using both absolute and relative WBC differential counts. Method correlation data from these samples were determined using EP Evaluator (Data Innovations LLC). Regression statistics, random error estimates, and systematic error (absolute and percent bias) were determined. **RESULTS:** Acceptance criteria included well-separated leukocyte scattergrams from both Sysmex XN-1000V and Siemens Advia 120 platforms. Regression values for automated neutrophil and lymphocyte count comparisons were very good to excellent ($R \geq 0.90$) for mice and rats. Eosinophil percent values showed higher variability between species ($R \geq 0.90$ for mice and $R \geq 0.80$ for rats) however, absolute values showed less agreement for rodents ($R \leq 0.76$). Microscopic manual methods correlated well with the automated platforms for both species ($R \geq 0.87$) for neutrophils and lymphocytes but monocytes and eosinophils showed less agreement ($R \leq 0.71$). Basophils were not observed in these rodent studies. This was likely due to low number of cells included. The analyzers showed similar correlation to manual counts for rodents with rats having higher correlation than mice. Agreement between analyzers was better than between each analyzer and manual counts likely due to the high number of cells processed by the analyzers compared to low number of cells counted manually. Percent bias data was generally ≤25% with the majority of comparisons showing less than 10% bias. Some differential count parameters having inherently low cell numbers (e.g. Mono%, Baso% and #Baso) had much higher systematic differences. **CONCLUSIONS:** The Sysmex XN-1000V performed well for samples from laboratory mice and rats for automated differential leukocyte counts. The Sysmex XN-1000V showed comparable performance and acceptable correlation to the Siemens Advia 120 results and to manual microscopic analyses. Based on these results, the Sysmex XN-1000V is suitable for use to support nonclinical studies in drug development.

Wednesday, August 1, 2018

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

Automation/Computer Applications

B-012

Implementation of a web-based Quality Control Management System for Clinical Chemistry Laboratories in a Standardized Healthcare System

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Background: As we continue expanding our laboratories services at Florida Hospital Healthcare System, additional tools were needed to monitor quality control in Chemistry Laboratories. The objective of implementing Unity Real Time QC Management system was to improve monitoring analytical performance metrics more efficiently and effectively. **Methods:** This study collected 6 months of quality control data for 12 networked laboratories, across fifteen Roche 6000, two Roche 8000, and three Roche Integras instruments**.** This data was evaluated against BioRad peer group and existing Sunquest quality control system. Manual selection of statistical process rules (SPC) were determined for each analyte. Fixed means/SDs or floating means/SDs were selected to use for SPC rule evaluation. Data review was performed by using standard Levey Jennings (LJ) charts, Multi LJ chart, Youden chart, and Yundt plot. Data Analysis grid reports provided side by side comparison between peer group and own laboratory, across multiple instruments, and between own laboratory and Florida Hospital peer group. Onsite training was offered by the vendor for superusers and computer based training was deployed to medical technologists involved in QC review before implementation. **Results:** Prior efforts to monitor analytical performance reviewing quality control in Sunquest system were done inefficiently. The quality control review was time consuming and only resulted in evaluating quality control using Levey Jennings charts. Unity Real Time QC management system simplifies quality control evaluation and provides multiple tools within the program to efficiently monitor performance trends for each analyte. This program allowed us to effectively identify quality problems by collating data into statistically significant charts and plots. In addition, it helped us identify instrument and assay performance changes over time and which variables play a role in these changes (reagent lot changes, calibrations, new QC lot changes, etc). The implementation of this software improved documentation of corrective actions in the laboratory. We also noted optimization of error detection without additional QC analysis and troubleshooting. **Conclusion:** The implementation of a web-based QC Management System in a large healthcare System provides flexible options for quality control evaluation. Unity Real Time offers a robust method for data comparisons helping us to meet regulatory requirements, allowing a high-level overview of networked instruments and standardized and centralized quality control data management for the entire system.

B-013

Performance Evaluation of Atellica IM 1600 Analyzer Assays at a Medical Laboratory

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Background: In our institution, studies were performed to assess the analytical performance of immunoassays (IM) for the Atellica® IM 1600 Analyzer with respect to verification of precision and linearity, and method comparison with Siemens Healthineers assays on ADVIA Centaur® XP System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material ranged up to six depending on the assay. For each assay, three replicates of each sample level were assayed. **Results:** Within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) IM CVs ranged from 1.3% to 5.3% and total (within lab) IM CVs from 1.7% to 10.3%. Linearity studies have been performed for all assays. Precision and method comparison studies are summarized below.

		Precision			Method Comparison
Atellica IM Analyzer Assay	**Units**	**Mean conc. (low, high)**	**Within run %CV(SD) (low, high)**	**Total %CV(SD) (low, high)**	**Atellica IM Analyzer vs. ADVIA Centaur XP System; Deming Fit**
Fer	ng/mL	15.06, 330.47	2.7(0.40), 1.8(5.90)	10.3(1.55), 5.3(17.38)	y=0.89x-0.59
VB12	pg/mL	192.40, 765.56	4.4(8.48), 1.3(9.65)	5.1(9.89), 2.1(15.71)	y=0.93x+60.6
VitD	ng/mL	27.05, 96.9	4.6(1.26), 2.0(1.98)	7.2(1.94), 4.3((4.20)	y=0.95x-2.12
iPTH	pg/mL	37.76, 814.27	1.6(0.59), 1.6(13.09)	2.1(0.80), 2.3(18.61)	y=1.07x-0.13
TnIH*	pg/mL	10.44, 5647.85	3.9(0.41), 1.1(60.42)	4.1(0.43), 2.4(134.46)	y=0.90x+0.39
BNP	pg/mL	46.90, 1887.03	2.8(1.32), 1.5(27.46)	5.8(2.74), 3.1(58.65)	y=1.13x+3.73
PSA*	ng/mL	0.14, 15.81	2.7(0), 1.9(0.31)	4.0(0.01), 2.6(0.41)	y=0.90x-0.06
CA 199	U/mL	21.06, 271.93	4.6(0.97), 5.3(14.34)	7.4(1.55), 9.7(26.46)	y=0.89x+11.9
AFP*	ng/mL	33.72, 248.68	3.0(1.02), 3.1(7.61)	3.4(1.14), 4.7(11.70)	y=0.91x-0.145
eE2	pg/mL	34.98, 950.32	4.5(1.57), 1.7(16.56)	7.3(2.54), 4.2(39.91)	y=0.96x-1.03
ThCG*	mIU/mL	5.75, 394.32	3.6(0.21), 2.1(8.19)	3.6(0.21), 2.8(10.91)	y=1.07x+3.46
PRGE	ng/mL	1.35, 22.21	4.5(0.06), 1.9((0.42)	5.5(0.07), 3.9(0.88)	y=1.19x+0.36
TSTII	ng/dL	20.43, 1065.93	3.2(0.66), 3.3(35.26)	4.3(0.88), 7.3(78.28)	y=0.89x-8.86

Conclusions: All assays tested on the Atellica IM Analyzer demonstrated good precision and correlation to the current ADVIA Centaur XP System assays. The precision results were consistent with manufacturer's claims. *Siemens Healthineers supported the study by providing systems, reagents, and protocols, and contributed to data analysis.

B-014

Assessment of Average Patient Results for the Use of a Patient Results-Based Quality Improvement Program

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Background: Patient results based quality improvement approach has been applied in clinical laboratories. The volume of the patient test results selected for analysis may play an important role in an accurate and efficient lab quality control (QC) program. The literature found had no clear guidelines on the quantity of results needed, ranging from 10 to 100 results. In this study, we retrieved and analyzed a large amount of patient data comparing the variation of the average test results with test volumes. **Methods:** Average patient results of comprehensive metabolic panel (CMP) and volume of tests in varying time periods were extracted from July 2015 to December 2017 via Viewics® program. For each period of time with corresponding test volume, the weighted average (wAVG) of test results, standard deviation (SD), coefficient of variation (CV) for each analyte were calculated using Microsoft Excel®. **Results:** CVs and test volumes of analytes for the periods of time are shown in the Table. The results show that increased volume of results in the periods of time correlated with decreased CV. For electrolytes (chloride, CO_2, potassium, and sodium) after daily period (volume ~780) there was no significant decrease in CV, and even hourly period (volume around 40) CVs are less than 5%. For basic metabolic panel (BMP), albumin, and protein after the weekly period (volumes ~5,500, ~3,500, and ~3,500, respectively) there is no appreciable decrease in CV. CVs of enzymes (ALP, ALT, AST) and bilirubin are generally much higher than other tests. **Conclusion:** The results of CV variation of average patient results we found in the study may guide in the better selection of the volume of patient results in an application of a patient results based quality improvement program, such as real time QC monitoring and review.

	Hour		Day		Week		Month		Quarter	
Analyte	CV	Volume	CV	Volume	CV	Volume	CV	Volume	CV	Volume
Albumin	13.7%	22	8.9%	515	3.1%	3,564	2.9%	16,065	3.1%	45,866
Bilirubin	134.0%	22	53.3%	519	12.9%	3,602	7.7%	16,375	6.1%	46,873
ALP	48.8%	21	14.9%	501	5.6%	3,478	5.0%	15,690	5.0%	44,957
ALT	167.7%	21	46.3%	503	16.6%	3,496	11.0%	15,755	8.7%	44,964
AST	518.1%	21	91.2%	503	22.7%	3,494	13.2%	15,746	10.6%	44,959
BUN	35.6%	33	11.6%	784	7.3%	5,605	6.0%	25,058	6.4%	71,696
Calcium	3.9%	33	2.2%	777	0.9%	5,567	0.7%	24,966	0.6%	71,364
Chloride	1.6%	33	0.5%	777	0.3%	5,563	0.2%	24,929	0.2%	71,272
CO2	5.4%	33	2.2%	777	2.2%	5,562	2.1%	24,927	2.0%	71,267
Creatinine	46.3%	33	14.8%	782	9.9%	5,597	2.8%	25,084	2.2%	71,698
Glucose	20.2%	33	5.5%	779	1.5%	5,583	1.0%	25,009	0.6%	71,486
Potassium	4.5%	33	1.4%	781	0.6%	5,593	0.5%	25,055	0.3%	71,611
Protein	8.7%	21	4.8%	499	1.6%	3,470	1.0%	15,665	1.0%	44,736
Sodium	1.1%	33	0.4%	785	0.6%	5,636	0.6%	25,218	0.5%	72,062

B-015

Utility of CEA kinetics in predicting recurrence of colorectal cancer in India in a tertiary cancer hospital.

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Incidence of colon cancers is on the increase in India. Routine testing of serum carcinoembryonic antigen (CEA) is performed in the laboratories as a marker for prognosis and prediction. It is also used as an adjunct to diagnosis with other clinical and imaging modalities. We retrieved serial data available on the electronic records from 2014 to 2016. A model was built using patients who had CEA values above 5 ng/mL but were currently stable after surgery with or without neoadjuvant chemoradiotherapy. The model was tested using a training dataset and the accuracy of prediction checked using a test dataset. A bi exponential model was built to predict CEA at time t. $CEA(t) = CEA(0)\, e^{-0.997.t} + CEA(t3)e^{(-0.6458.t)}$. Half-life for patients without recurrence was 20.2 (±3) days as against 48.7 (±8) days for patients with recurrence. The rate of clearance of CEA in the former group was 141.22 ng/mL*month respectively whereas the group with recurrence has a clearance rate of 102 ng/mL*month respectively. The accuracy of prediction was determined to be 64.3%. The model has the ability to predict cases that can present with recurrence. Further clinical validation needs to be carried out. Currently a web based application has also been made available.

B-016

Telemicroscopy: An innovative solution to an age-old problem

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Background: Telemicroscopy is the digitizing of images from a microscope and transmitting those images to another location via the internet. Multicare Health System has adopted this technology in hematology and microbiology to provide real-time support for satellite hospitals and remote clinics, especially in the areas of interpreting challenging cells on a differential and identifying organisms on a Gram stain. This telemicroscopy model allows the Core Lab to see exactly what the outlying locations are seeing in real-time and provide feedback in making an accurate decision on differentials and Gram stains, thereby improving patient care. **Methods:** When a technologist at an off-site location is looking at a slide and needs a second-opinion consultation from an experienced technologist, they call the Core Lab. A ProScope 5MP Microscope Camera (Bodelin, Wilsonville, OR), under $500, is inserted into an ocular of a Nikon or Leica microscope at the off-site location and the Micro Capture Software (Bodelin, Wilsonville, OR) is opened. When the Core Lab receives the consult request, a web conference using GoToMeeting (Logmein,Boston, MA) is initiated. The Core Lab technologist will then "Share Your Screen" from the GoToMeeting, and "Change Presenter to" the off-site location. This allows the Core Lab to dynamically look at the subject under the off-site microscope. The protected health information (PHI) are discussed over the phone, and not documented by either software. With the real-time video streaming and discussion of the clinical case, the Core Lab can give an accurate second-opinion consultation. **Results:** The technologist from the Core Lab would see images from the ProScope 5MP Camera through the GoToMeeting software. **Conclusion:** This technology offers a relatively inexpensive option for locations that do not have the clinical expertise that the Core Lab has in analyzing slides. Moreover, telemicroscopy offers a valuable educational opportunity with real-time feedback on difficult cells or organisms.

B-017

Fully Automated DNA Isolation and NGS Library Preparation

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Background: Although the total cost of next generation sequencing (NGS) continues to decline, considerable time and cost is required to isolate the DNA and prepare sequence-ready libraries from a variety of different human tissue sources. The goal of the present study was to simplify and fully automate these crucial steps in order to reduce the time and cost of as well as the level of training required to complete these critically important tasks. **Methods:** A microfluidic cartridge and workstation were designed to fully automate the isolation of DNA and then generate sequence-ready DNA libraries from a variety of different human sources, including buccal swabs, formalin fixed paraffin embedded (FFPE) tissue blocks, fresh frozen tissue and blood. The workstation's software was programmed to isolate the DNA and then prepare sequence-ready libraries using reagents from a number of different Illumina NGS library prep kits (including Nextera DNA Preparation, Nextera XT, Nextera Flex and AmpliSeq). Finally, the resulting DNA libraries were sequenced on HiSeq 2500 or MiSeq instruments and sequence data and quality metrics analyzed. **Results:** The automated workflow yielded DNA of sufficient quantity and quality to allow the microfluidic system to be programmed to prepare sequence-ready NGS libraries using a variety of Illumina library prep kits. The isolated DNA was of high quality, based on A260/A280 and A260/A230 ratios, and was then automatically processed by transposome-mediated fragmentation, followed by low cycle PCR to integrate the required Illumina adaptors and index codes. The libraries, purified by bead-based methods, had size distributions that were optimal for Illumina sequencing and were clean of short adaptor sequences and ligated adaptors. Sequence data derived from the automatically prepared libraries was able to be effectively aligned against the reference genome with depth and uniformity of coverage that exceeded that obtained by manual methods. Other quality metrics obtained from the sequencing runs were also excellent, including high Q30 scores, pass filter scores, and low error rates. **Conclusion:** The ability to automatically isolate DNA and prepare sequence ready libraries on a single instrument that requires very little "hands on" effort will reduce the time, cost and effort of next generation sequencing. Moreover, the ability to process a range of tissue types will allow a broad application of NGS including detection of genetic variants in germ line and somatic cells. Finally, as sequencing costs continue to decline, the percent of total costs associated with sample preparation has gone up. Therefore, simplifying and automating combined DNA isolation and library preparation will not only reduce the total time and cost of these prerequisite steps, but also appeal to third party payers as the clinical utility of NGS data justifies its diagnostic applications.

B-018

Autoverification Implementation through Middleware in a Large Hospital Core Chemistry Laboratory: Gains in Quality and Efficiency

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Background: Much of healthcare is transitioning to value-based care, and the laboratory is not exempt. Autoverification (i.e. result release without human intervention) implementation through middleware was sought to heighten the quality of laboratory results while decreasing cost (value=quality/cost). The autoverification rules were removed from the laboratory information system (LIS; Sunquest), and implemented in middleware (Data Innovations) between 4 Cobas 8000 lines (Roche Diagnostics) and the LIS. The Rules Package Guidance Document (Roche Diagnostics) supplied the foundation which was then customized: analytical measurement ranges, clinical reportable ranges, critical values, delta values, specimen integrity rules, and LIS formatting. After installation, the middleware-based autoverification rules were validated via electronic simulated testing (i.e. dry testing) and specimen testing (i.e. wet testing). The laboratory services a 1400-bed hospital, and supports outreach and outpatient centers. **Methods:** To observe any gains in value, the following metrics were observed pre- and post-middleware autoverification implementation: autoverification rates, manual reviewers needed, and ED STAT specimen turnaround time. The autoverification data was collected for 48 days before and after implementation. ED turnaround time (i.e. receipt in lab to result release in the LIS) was averaged for 6 months before and after implementation. **Results**: Overall, the autoverification rate saw a dramatic increase from 63% to 92% (**table-1**). The increase in the autoverification rate allowed the reduction of 1 manual reviewer per weekday (1 full time equivalent). In addition to the efficiency increase (i.e. reduction

of cost), gains in quality were also noted. In combination with other initiatives, the ED STAT specimen turnaround time decreased by 3 minutes. The number of specimen quality rules were increased (e.g. delta checks), resulting in the timely detection of analytical issues. **Conclusion**: An increase in the value of laboratory tests results were observed via the implementation of autoverification through middleware.
Table-1: Autoverification Rates for 48 Days Before and After Middleware Autoverification Implementation

	Pre-Implementation		Post-Implementation	
	Autofile Rate (%)	**Total orders**	**Autofile Rate (%)**	**Total Orders**
Basic Metabolic Panel	69	43674	94	44644
Comprehensive Metabolic Panel	60	74760	89	69455
Hepatic Function Panel	71	5556	87	5442
Renal Function Panel	62	3894	90	5575
TSH	74	25242	>99	27299
FT4	70	7379	>99	10042
Overall	63	312913	92	407006

B-019

Performance Evaluation of Atellica CH 930 Analyzer Assays at a Medical Laboratory

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Background: In our institution, studies assessed the analytical performance of clinical chemistry (CH) and plasma protein assays for the Atellica® CH 930 Analyzer with respect to verification of precision and linearity, and method comparison with Siemens Healthineers assays on the ADVIA® 1800 System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges. The number of levels of linearity material (LGC Maine Standards) ranged up to six depending on the assay. For each assay, three replicates of each sample level were assayed. **Results:** Within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) CVs ranged from 0.2% to 5.3% and total (within lab) CVs from 0.4% to 5.3%. Linearity studies have been performed for all assays. Precision and method comparison studies are summarized below.

	Precision			Method Comparison		Precision			Method Comparison
Atellica CH 930 Analyzer Assay	Mean conc. (low, high)	Within run %CV (SD) (low, high)	Total %CV (SD) (low, high)	Atellica CH 930 Analyzer vs. ADVIA 1800 System	Atellica CH 930 Analyzer Assay	Mean conc. (low, high)	Within run %CV (SD) (low, high)	Total %CV (SD) (low, high)	Atellica CH 930 Analyzer vs. ADVIA 1800 System
GluO, g/L	0.58, 3.44	0.9(0.0), 0.5(0.0)	2.0(0.0), 1.3(0.0)	y=0.99x + 0.01	CRP_2, mg/L	6.7, 51.24	1.4(0.1), 0.8(0.4)	2.8(0.2), 1.2(0.6)	y=1.03x - 1.23
UN_c, mg/dL	15.18, 71.88	2.5(0.4), 0.5(0.4)	3.3(0.5), 1.3(0.9)	y=1.01x + 1.22	RF, IU/mL	23.96, 43.49	2.2(0.5), 0.8(0.4)	3.5(0.9), 3.9(1.7)	y=0.98x + 2.00
ECre_2, mg/L	8.13, 68.32	2.3(0.2), 1.2(0.8)	3.3(0.3), 1.2(0.8)	y=0.96x - 0.03	Na, mmol/L	115.07, 157.43	0.4(0.5), 0.2(0.3)	1.3(1.5), 1.6(2.5)	y=1.04x - 3.63
AST, U/L	46.37, 291.63	2.7(1.3), 1.3(3.9)	3.1(1.5), 1.8(5.1)	y=0.99x + 6.40	K, mmol/L	2.47, 7.33	0.4(0.0), 0.2(0.0)	1.4(0.0), 1.6(0.1)	y=1.04x - 0.16
ALT, U/L	31.57, 213.43	5.3(1.7), 1.6(3.4)	5.3(1.7), 2.9(6.1)	y=1.03x - 2.81	Cl, mmol/L	77.58, 120.80	0.3(0.2), 0.2(0.3)	0.9(0.7), 1.4(1.6)	y=1.00x + 1.50
TP, g/L	39.86, 70.36	4.5(1.8), 0.3(0.2)	4.6(1.9), 0.4(0.3)	y=0.93x + 2.02	Crea_2, mg/L	10.15, 66.43	1.4(0.1), 0.6(3.6)	1.8(0.2), 1.0(0.6)	Not done
Trig, g/L	0.89, 2.13	0.7(0.0), 0.7(0.0)	2.3(0.0), 1.1(0.0)	y=0.94x - 0.017	Ca, mg/L	55.03, 133.36	1.1(0.6), 0.5(0.7)	1.6(0.9), 0.6(0.9)	Not done
TBil_2, mg/L	6.18, 73.81	1.1(0.1), 0.3(0.2)	4.6(0.3), 3.4(2.5)	y=1.04x - 0.24	Alb, g/L	2.61, 4.47	1.5(0.04), 1.2(0.06)	1.6(0.0), 1.6(0.1)	Not done
UA, mg/L	34.52, 96.68	0.5(0.9), 0.4(0.4)	0.9(0.3), 0.7(0.7)	y=1.01x - 0.43	B2M, mg/L	678.33, 3086.4	2.2(15), 0.8(26)	2.5(17), 0.9(27)	Not done

Conclusions: All assays tested on the Atellica CH 930 Analyzer demonstrated good precision and correlation to the current ADVIA 1800 System assays. The precision results were consistent with manufacturer's claims. * Siemens Healthineers supported the study by providing systems, reagents and protocols, and contributed to data analysis.

B-020

Performance Evaluation of the Atellica IM Thyroid Stimulating Hormone 3-Ultra Assay and Impact of Biotin

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Background: The Atellica® IM Thyroid Stimulating Hormone 3-Ultra (TSH3-UL) assay is for in vitro diagnostic use in the quantitative determination of thyroid-stimulating hormone (TSH, thyrotropin) in human serum and plasma (EDTA and heparin) using the Atellica® IM 1600 Analyzer. The study objectives were to demonstrate acceptable method comparison between the Atellica® IM TSH3-UL assay, ADVIA® Centaur TSH3-UL assay, and Roche cobas® TSH assay; and evaluate the potential impact of biotin interference on the Atellica IM TSH3-UL Assay and on the Roche cobas TSH assay. **Methods:** The Atellica IM TSH3-UL Assay is a third-generation assay that employs anti-FITC monoclonal antibody (mAb) covalently bound to paramagnetic particles, a FITC-labeled anti-TSH capture mouse mAb, a tracer consisting of a proprietary acridinium ester and an anti-TSH mouse mAb conjugated to bovine serum albumin for chemiluminescent detection. The TSH3-UL does not employ the biotin-streptavidin complex in the assay design, so should have minimal biotin interference. Precision and linearity studies were performed by EP15-A3 and EP06-A, respectively. Method comparison results were performed according to CLSI EP09-A3. Sample types included adult serum and plasma (EDTA or heparin). Samples (1065) were from the site's routine thyroid testing, across the range of the assay and were tested on the Atellica IM 1600 Analyzer, ADVIA Centaur® XP System, and Roche cobas® analyzer in singleton. In case of discrepant results between the assays tested on the different analyzers, samples were repeat-tested on all analyzers. If discrepancies remained, the clinical status of the patient, FT4 and FT3 results and presence of biotin treatment were investigated. Serum samples spiked with biotin (30, 500 ng/mL) and un-spiked samples (controls) were run in duplicate with both the Atellica IM TSH3-UL Assay and Roche cobas TSH assay. **Results:** Precision and linearity studies agreed with the manufacturer's claims for the Atellica TSH3-UL assay: The assay was demonstrated to be linear from 0.0 to 120.05uIU/mL (y=1.018x+0.006). Within run repeatability CV%(SD)s for concentrations of 0.01, 0.70, 5.84, and 29.95 uIU/mL were 5.5%(0.00), 1.3%(0.01), 1.3%(0.08), 1.3%(0.39); within lab (total) CV%(SD)s were 6.3%(0.00), 2.7%(0.02), 2.4%(0.14), and 2.0%(0.61), respectively for the Atellica TSH3-UL assay. Method comparison of Atellica IM TSH3-UL Assay vs. Roche cobas TSH assay showed a

regression slope of 0.92, intercept of 0.002 and correlation coefficient (r) of 0.982; Method comparison of Atellica IM TSH3-UL Assay vs. ADVIA Centaur TSH3-UL assay showed a regression slope of 1.06, intercept of -0.001 and correlation coefficient (r) of 0.994 . The Atellica IM TSH3-UL assay recovered 5.28uIU/mL (negative control) vs. 5.33uIU/mL (500ng/mL biotin spiked); while, the Roche cobas TSH assay recovered 5.28uIU/mL (negative control) vs. 0.10uIU/mL (500ng/mL biotin spiked) thereby demonstrating biotin interference in the Roche cobas TSH assay. **Conclusions:** Precision and linearity studies agreed with the manufacturer's claims on the Atellica TSH-3 UL assay. Method comparison of the Atellica IM TSH3-UL Assay to the ADVIA Centaur TSH3-UL assay and Roche cobas TSH assay demonstrated good agreement. The Atellica IM TSH3-UL Assay showed <10% bias at a biotin concentration of 500ng/ml. ***** Siemens Healthineers supported the study by providing systems, reagents, and protocols, and contributed to data analysis.

B-021

Reducing turnaround time for ED critical results reporting through advanced LIS rules

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Reporting of critical values on Emergency Department specimens requires both timely and accurate resulting. Despite evidence that repeat testing may not be necessary for critical values within the analytic measurement range (AMR), how altering this long-standing practice reduces laboratory turnaround time (TAT) has not been reported. Moreover, how implementation of advanced laboratory information system (LIS) rules to ensure the accuracy of reported critical results has not been reported. To address this, our laboratory implemented advanced LIS rules to allow for the reporting of critical values to the ED prior to repeat testing. We assessed results before and after LIS implementation to confirm that repeat results were within the allowable error. LIS rules were established using middleware software (Data Innovations, Burlington, Vermont). Briefly, samples within the reference interval were reported via normal autoverification rules. If a sample was outside of the reference interval, it was either in the absurd range, or the critical range. Any samples within the absurd range had all results from the specimen withheld, even if other results were normal. These were checked by a medical technologist prior to release of the sample or a redraw of the specimen was ordered. All other values within the critical range were immediately called to a patient caregiver. To ensure accuracy of laboratory results, specimens with critical values were repeated. If there was a significant change from the first result, it would immediately be called to the ED. Normal autoverification rules for icterus, hemolysis index, triglycerides and delta checks were applied to each sample. We also analyzed the effect of advanced LIS implementation on TAT. In total, we examined 437 samples with critical/absurd values within the AMR from ED patients from three separate periods in 2013-2016. In the ED, repeat testing on samples with critical values demonstrated 99.5% precision, as only one sample was outside the acceptable CAP/CLIA variation upon repeat testing. This was in contrast to non-ED floors and outpatient clinics, which had a surprisingly low discordance of 5.5% between original and repeated results. Moreover, advanced LIS implementation significantly reduced additional TAT for ED specimens. The mean additional TAT for critical results was 14.2 minutes in 2013 prior to advanced LIS rule implementation. This was reduced to 11.9 minutes and 10.8 minutes in 2014 and 2016 respectively following LIS implementation. Importantly, samples taking longer than 25 minutes of additional TAT were reduced from 11.7% to 1.2% following advanced LIS implementation. We conclude that implementation of advanced LIS rules allows for immediate reporting of non-absurd critical values prior to repeat in specimens from the ED. Moreover, advanced LIS rules reduce both mean TAT and the proportion of long, additional TAT greater than 25 minutes. Together, these findings demonstrate the clinical utility of implementing advanced LIS rules to automated analyzers to improve laboratory efficiency while reducing turnaround time, ultimately leading to improved patient care.

B-022

Performance Evaluation of Immunoassays on the Atellica IM 1600 Analyzer

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Background: Studies were performed to assess the analytical performance of immunoassays (IM) for the Atellica® IM Analyzer with respect to verification of precision, linearity, detection limit, and method comparison with Siemens Healthineers lab assays on the ADVIA Centaur® XPT System. **Methods:** Precision verification was performed according to EP15-A3, method comparison by EP09-A3, linearity by EP06-A, and detection limit by EP17-A2. For precision, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run each day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed on 44 to 50 serum samples covering each assay range, from low to high. The number of levels of linearity material ranged up to nine depending on the assay. For each assay, three replicates of each sample level were assayed. Two LoB and two LoD samples were processed in the same run running 4 replicates each day for 3 days for a total of 24 blank measurements for each assay. Two LoQ samples were processed in one run per day, five replicates per run, for five days, for a total of 25 replicates for each sample. **Results:** All within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) CVs ranged from 1.2% to 6.8% and total (within lab) CVs from 1.7% to 13.7%. Linearity and verification of detection capability studies were performed for all assays. Precision and method comparison studies are summarized below.

		Precision			Method Comparison
Atellica IM Analyzer Assay	**Units**	**Mean conc. (low, high)**	**Within run %CV(SD) (low, high)**	**Total %CV(SD) (low, high)**	**Atellica IM Analyzer Assay vs. ADVIA Centaur XPT System Assay**
Fer	ng/mL	22.65, 328.74	4.7(1.07), 3.3(10.83)	6.3(1.42), 4.3(14.18)	[b]y=1.05x-1.09
VitD	ng/mL	25.38, 87.10	3.8(0.96), 3.1(2.69)	11.0(2.79), 5.5(4.82)	[b]y=0.89x+0.85
iPTH	pg/mL	40.45, 840.44	2.1(0.83), 1.9(16.10)	5.1(2.05), 4.1(34.57)	[b]y=1.05x-0.56
PSA*	ng/mL	0.14, 15.13	3.6(0.00), 2.9(0.44)	3.8(0.01), 4.9(0.74)	[b]y=0.93x-0.02
AFP*	ng/mL	32.49, 243.34	4.6(1.48), 3.8(9.13)	5.6(1.80), 3.8(9.13)	[a]y=1.01x-1.36
CEA	ng/mL	2.32, 36.38	6.8(0.16), 2.9(1.07)	13.7(0.32), 5.4(1.98)	[b]y=1.02x-0.35
eE2	pg/mL	37.67, 970.31	6.8(2.54), 2.0(19.51)	8.8(3.1), 3.6(34.45)	[b]y=0.92x-8.5
ThCG*	mIU/mL	5.52, 369.88	2.3(0.13), 2.0(7.45)	4.8(0.26), 2.3(8.65)	[b]y=0.96x+2.81
PRGE	ng/mL	1.34, 23.45	4.0(0.05), 2.6(0.62)	4.7(0.06), 3.9(0.91)	[b]y=1.01x+0.04
TSTII	ng/mL	0.12, 11.57	5.0(0.01), 4.0(0.46)	5.1(0.01), 5.5(0.64)	[b]y=0.94x-0.01
TSH3UL	uIU/mL	0.01, 26.99	6.2(0.00), 1.8(0.48)	8.1(0.00), 2.8(0.75)	[a]y=0.95x+0.14
FT4	ng/dL	1.10, 3.65	2.0(0.02), 1.2(0.04)	3.6(0.04), 2.3(0.08)	[b]y=1.08x+0.03 ng/dL

[a]Passing&Bablock Fit; [b]Deming Fit

Conclusions: All immunoassays tested on the Atellica IM Analyzer demonstrated good precision and correlation to the current ADVIA Centaur XPT System assay. The precision results were consistent with manufacturer's claims. * Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis.

B-023

Performance evaluation of chemistry and plasma protein assays on the Atellica CH 930 Analyzer.

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Background: Atellica® CH 930 is the new analyzer from Siemens Healthineers. In our Hospital, we have assessed the analytical performance of chemistry assays (CH) listed in Table 1. The aim of the study was to evaluate precision, detection limit (LoD) and linearity of Atellica as well as a method comparison (MC) with ADVIA® Chemistry XPT and Dimension Vista® System. **Methods:** Precision, linearity, LoD, and MC were performed following EP15-A3, EP06-A, EP17-A2 and EP09-A3 respectively. For Precision quality control materials (QC) and sample pools were tested, as one run per day with five replicates, for five days (25 total replicates/sample/assay). The number of levels of linearity mate-

rial (LGC Maine Standards) ranged up to six depending on the assay. For each assay, three replicates per level were assayed. Two detection of blank (LoB) and two detection limit (LoD) samples were processed in the same run with four replicates each day for three days (24 measurements/assay). Two limit of quantification (LoQ) samples were processed in one run per day with five replicates, for five days, (25 replicates/sample). Finally, in the MC we assessed 40 serum samples per assay and they were processed by legacy (reference system) and Atellica in the same day. **Results:** Atellica CH assays showed a good linearity per the Maine Software Data Reduction Program. LoB, LoD and LoQ were successfully verified for all CH assays. Precision study and MC results are summarized in Table 1.

Atellica CH 930 Analyzer Assay	Mean conc. (low, high)	Within run %CV (SD) (low, high)	Total %CV (SD) (low, high)	MC Atellica CH vs ADVIA Chemistry XPT	Atellica CH 930 Analyzer Assay	Mean conc. (low, high)	Within run %CV (SD) (low, high)	Total %CV (SD) (low, high)	MC Atellica CH vs. ADVIA Chemistry XPT
GLUH_c mg/dL	60, 354	0.8 (0.5), 0.3 (1.1)	2.7 (1.7), 1.4 (4.9)	y=0.96*X - 1.60	TBil_2 mg/dL	0.6, 7.7	7.2 (0.04), 0.4 (0.03)	7.2 (0.04), 0.5 (0.04)	y=0.99*X + 0.02
UN_c mg/dL	7.3, 34	1.3 (0.10), 0.6 (0.20)	1.7 (0.12), 1.1 (0.38)	y=1.06*X - 1.39	Na mmol/L	115, 160	0.5 (0.62), 0.3 (0.45)	0.5 (0.62), 0.4 (0.60)	y=1.03*X - 5.31
Crea_2 mg/dL	0.74, 6.29	1.5 (0.01), 0.5 (0.03)	1.7 (0.01), 0.8 (0.05)	y=1.02*X - 0.01	K mmol/L	2.62, 7.4	0.3 (0.01), 0.2 (0.01)	0.4 (0.01), 0.3 (0.02)	y=1.00*X - 0.15
Ca mg/dL	5.6, 13.5	1.2 (0.06), 0.6 (0.08)	1.8 (0.10), 1.1 (0.15)	y=0.99*X - 0.08	Cl mmol/L	78.3, 121	0.6 (0.44), 0.3 (0.32)	1.0 (0.76), 0.3 (0.36)	y=1.02*X - 3.46
AST U/L	43, 295	1.4 (0.62), 0.4 (1.30)	2.4 (1.01), 0.8 (2.45)	*	APO A mg/dL	110.4, 149.8	1.0 (1.07), 2.0 (3.05)	4.9 (5.35), 3.9 (5.90)	y=1.10*X - 4.44
ALT U/L	31, 218	3.4 (1.06), 0.6 (1.28)	5.1 (1.58), 1.1 (2.40)	*	APO B mg/dL	39.7, 148.5	3.5 (1.37), 1.2 (1.78)	6.0 (2.38), 5.8 (8.58)	y=0.93*X - 1.13
TP g/dL	4.1, 7.2	0.9 (0.04), 0.3 (0.02)	1.2 (0.05), 0.6 (0.04)	y=1.02*X + 1.01	B2M mg/L	0.70, 3.08	1.8 (0.01), 2.1 (0.07)	2.0 (0.01), 2.2 (0.07)	y=1.11*X + 0.16 (vs. Vista)
ALB_c g/dL	2.6, 4.5	1.6 (0.04), 1.0 (0.04)	1.9 (0.05), 1.0 (0.04)	y=1.05*X + 0.43	CRP_2 mg/dL	0.67, 5.17	2.5 (0.02), 0.8 (0.04)	2.9 (0.02), 1.4 (0.07)	y=1.00*X - 0.05
Trig mg/dL	94, 214	0.4 (0.4), 0.5 (1.0)	1.1 (1.0), 0.8 (1.7)	y=0.99*X + 0.20	hsCRP mg/L	0.97, 7.86	2.9 (0.03), 1.1 (0.09)	6.4 (0.06), 1.5 (0.12)	y=0.96*X + 0.29

*Not done.

For MC all assays yielded p values ranging from 0.995 to 1.000 **Conclusions:** All the assays tested on the Atellica CH 930 System demonstrated good precision, linearity LoB, LoD and LoQ. Precision results were consistent with manufacturer's claims. Finally, the method comparison between Atellica CH and Legacy systems (ADVIA Chemistry XPT and Dimension Vista) was satisfactory with a very good correlation for all assays tested.

B-024

Multicenter Throughput Study of the Atellica®Solution with Common Immunochemistry and Clinical Chemistry Panels

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Objective: To assess the throughput of different configurations of the Atellica® Solution using the same experimental protocol on a set of predefined test panels across multiple sites. **Methods:** Five different panels of tests with predefined similar profiles were assessed using 400 serum samples across three sites to observe throughput capabilities in a 1 hour period. The timing period commenced when the first sample was loaded into the Atellica® Sample Handler and ended after the last result of the last tube was available. All results in process were allowed to complete and the time of completion was noted. The test panels were:

A. Na, K, Cl, Glucose, Urea, Creatinine
B. Na, K, Cl, Total Protein, Calcium, ALT
C. TSH, HIV Ag/Ab Combo (CHIV)
D. TSH, hCG
E. hCV, Syphilis, HBsAg II, CHIV

The Atellica Solution configurations varied by site

- Laboratory 1: one Atellica Sample Handler, one Atellica® CH 930 Analyzer, and one Atellica® IM 1600 Analyzer
- Laboratory 2: one Sample Handler with two Atellica CH 930 Analyzers and two Atellica IM 1600 Analyzers. (Note: only one of each analyzer was used to ensure the like-for-like comparison.)
- Laboratory 3: one Atellica Sample Handler and two Atellica IM 1600 Analyzers (Note: only panels C, D, and E were run and only one analyzer was used to ensure the like-for-like comparison.)

Results

Table 1. Throughput observed across the study sites

	Laboratory 1		Laboratory 2		Laboratory 3	
Panel	**Tests initiated in first hour**	**Results per hour after first 30 min[a]**	**Tests initiated in first hour**	**Results per hour after first 30 min**	**Tests initiated in first hour**	**Results per hour after first 30 min**
A (CH)	**1239**	**1100**	**1230**	**1170**	**NT[b]**	**NT**
B (CH)	**1236**	**1051**	**1230**	**1081**	**NT**	**NT**
C (IM)	**329**	**231**	**288**	**226**	**276**	**246**
D (IM)	**397**	**346**	**316**	**304**	**413**	**408**
E (IM)	**319**	**203**	**287**	**225**	**263**	**239**

[a]This metric accounts for system throughput after initial workload buildup. [b]Not tested

Conclusions: Various configurations of the Atellica Solution consistently provide high throughput for a varying profile of test requests and is unaffected by the test mix of chemistry tests or immunoassays. Siemens Healthineers supported the study by providing systems, reagents, protocols and contributed to data analysis

B-025

Flowing mass spectrometry data to the LIS through a locally-developed data-management interface

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Background: With its superior sensitivity and specificity, mass spectrometry assay is increasingly being used in the clinical laboratories for identification and quantification of endogenous and exogenous compounds. Unlike most automated chemistry analyzers, the complex data generated from the mass analyzer is not in a LIS readable format. In addition, an electronic interface that streamline data flow and analysis between the instrument and laboratory information system (LIS) is usually not provided by the instrument or LIS vendors. Therefore, the large volume of the data rapidly generated from these instruments is usually manually entered into the LIS by the laboratory technicians. This manual data entry is challenging in modern clinical laboratories that are equipped and staffed for high-throughput, high complexity, and rapid turn-around of laboratory results in order to best serve the complicated patient mix. To overcome this hurdle, we aimed to develop an interface between our AB SCIEX mass spectrometer and our LIS (Sunquest Information Systems). **Methods**: We developed our own electronic interface utilizing Cresco, a Java-based agent framework. The interface was designed to process text files exported from Analyst software, which interfaces with a AB SCIEX 4500 triple quadruple mass spectrometer. Input records are checked against expected analyte values and an output report, in a format that the Sunquest can recognize, is generated. Network file systems are used to facilitate transfer of analytical data between the instruments, record archive, and the LIS. Interface implementation and data transfer validation as well as training of technologists to use the interface were performed by the Special Chemistry and Laboratory IT sections of the laboratory. The technologists were trained as to data verifications as part of the result acceptance process. **Results**: The estimated time for the technologist for patient/control sample data entry, assay results data transfer, and result verification was reduced from 90 seconds /specimen to under 15 seconds/specimen. Sample identification, results data entry errors and omissions were eliminated. The filing of the result in Sunquest generated an electronic record of the technologist performing the assay runs and data management. **Conclusion**: Development and implement of a data management interface for complex chromatography instruments in clinical laboratories has resulted in a

rapid, accurate, verifiable information transfers between our instrument and LIS. This has eliminated manual data entry that is error-prone and has unblocked the bottleneck in the application of mass spectrometry assays with improved workflow.

B-026

Validation of Procalcitonin Assay on Abbott Architect i1000

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Background: Sepsis, the 10th leading cause of death in the United States is the precursor to life threatening septic shock, which has a high mortality rate. However, symptoms of sepsis are not specific making it difficult to obtain early diagnosis resulting in delay of proper therapy. Procalcitonin and C-reactive protein are used as clinical biomarkers for sepsis but procalcitonin has been shown to be more sensitive and an early marker in monitoring septic shock. Food and Drug Administration cleared the use of B.R.A.H.M.S. PCT assay (Biomerieux, France) to help providers predict the likelihood of patient dying or if the patient's condition is worsening due to sepsis. In early 2017 FDA expanded the use of this assay to aid clinicians identify when antibiotic treatment should be initiated and halted. However, guidelines in pediatrics are still evolving. We anticipate that the expanded use of procalcitonin would result in an increase in utilization of this test, therefore validation of B.R.A.H.M.S. PCT assay on an automated, high throughput platform would be beneficial. **Methods:** The guidelines by Clinical and Laboratory Standards Institute (CLSI) EP5-A2 document was adopted in performing the validation. Precision and reportable ranges on Architect i1000 were performed. Manufacturer's instructions was followed, including quality control, calibration, calibration verification, and related functions. Procalcitonin in patient samples (N = 32) were measured in Mini Vidas and in Architect for comparison. Furthermore, we also tested the effect of major sources of intereferents such as free hemoglobin (12 g/L), triglycerides (12.43 mmol/L) and bilirubin (500 µM). Statistical analyses were performed using EP Evaluator software. **Results:** Architect method showed good precision with percent coefficient of variation (% CV) < 3.5% for both inter-assay and intra-assay compared to %CV < 6.5% for Mini Vidas. The analytical range in Architect was determined to be 0.02 - 100 ng/mL with a clinical reportable range of 0.02 - 1000 ng/mL. Statistical analysis showed that the two assays have good correlation (r >0.99), slope of 1.023, and intercept of -0.760. The calculated bias is -7.435%, indicating that Architect results on average is about 7% less than the results obtained on Mini Vidas. Pooled-serum spiked with 500 µM bilirubin showed the highest bias of about 6.5%. **Conclusion:** Validation results for Architect B.R.A.H.M.S. PCT assay revealed excellent precision and accuracy. The turn-around time for both platforms were the same (20 minutes), however in contrast to Mini Vidas, Architect offers automated pipetting of samples and can perform multiple assays. Mini Vidas is exclusively used for procalcitonin testing. Therefore, shifting the test to Architect will help streamline the process and eliminate manual pipetting of samples into reaction wells. These results indicated that Architect B.R.A.H.M.S PCT assay can be used for diagnostic purposes in clinical laboratories.

B-027

Use of a Contemporary TLA System in A Busy Clinical Chemistry Lab

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Background: With increasing workload, the staffs find that they have difficulties to achieve the required Turn-Around-Time(TAT) target of 60 min for AE and out-patient samples. The staffs also feedback that they have no time for administrative work and are physically exhausted due to making numerous trips to send samples to other analyzers in the section. **Methods**: 1.Map the Current Processes by on-site observation. 2. Identify Wastes in the Process Map (8 Wastes of Lean). 3. Reduce Complexity Lean Thinking/Streamline/Simplify/Eliminate/Technology). 4. Map the Future New Processes. The key members of the biochemistry team met up for 2 half days in January 2015 to map out the current process steps. Manual processes were identified and after brainstorming for improvements, it was agreed that the tests in the 3 system required consolidation and manual pre-analytics steps need to be automated. A total laboratory automation (TLA) system (cobas 8100, Roche Diagnostics) that can automate the manual pre- and post-analytic processes from specimen centrifugation, specimen repeat/rerun with dilution, refrigerated storage and sample disposal was chosen. Installation was carried out in phases as it was same site replacement of the old systems. Phase 1: Installation of 2 lines of analytics (cobas 8000 - Chem,Chem,Ecl,Ecl) from February-June 2016. Phase 2 : Installation of the pre-analytics unit (cobas 8100 with 3 centrifuges, decapper, aliquoter, sample buffer unit). Phase 3: Installation of the post-analytics storage system (cobas p501). **Results:** Before Implementation: The three areas that staffs of the Biochemistry Section needs to go is the DXC, cobas and Architect. There are in total 23 process steps to perform testing for a specimen in Biochemistry Section. For every trip from DXC to cobas is 23m and every trip made to Architect from DXC is 16.5m. To facilitate parallel workflow process, manual aliquots of the specimen is required. There could be approximately 455 aliquots to make in a day. After Implementation: The new process is mapped and the number of steps was reduced from 23 to 3 steps. The walking distance per trip is reduced from 23m to 7m. No manual aliquots were required as it is now automated. The new system brought an increase in staff satisfaction and morale as there is now more walkaway time to perform other tasks. The TAT achievement rate of 60 minutes (from sample receipt in the LIS to result reporting) for troponins request from AE before the new automated work processes from Jan to April 16 was 96.5% (Jan 16), 95.9% (Feb 16), 96.0%(Mar 16) and 94.0%(Apr 16). With the new processes, the TAT achievement rate was improved to 97.2%(Oct 16), 97.4%(Nov 16), 98.1%(Dec 16) and 97.6% (Jan 17). (Mann-Whitney test: p - 0.0286) **Conclusion:** The new system brought an increase in staff satisfaction and morale as there is now more walkaway time to perform other tasks. The TAT for patient's sample showed improvement and there is also reduce variations in the manual process by replacing it with automated process. The team will be exploring ideas (i.e. PST specimen type) to improve the TAT for outpatient's sample.

B-028

Data quality of Arkansas clinical data repository (AR-CDR)

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Background:
Researchers use clinical data collected at hospitals to conduct clinical studies. Results of these clinical studies depend on the quality of collected data. UAMS hospital has two data sources Arkansas clinical data repository (AR-CDR) and Epic system. (AR-CDR) is a rich clinical data source that regularly receive data feed from Epic, Regional programs, and other data sources. The evaluation of data warehouse quality is crucial to ensure the accuracy of clinical research results.
Methods:
We randomly selected the first one hundred diabetic patients, type 1 (ICD 10; E10) or type 2(ICD10; E11), from data warehouse who had visits between June 2016 and December 2016.We conducted a manual chart review of patients from EPIC system using the MRN, (patient ID number), as a primary key. For this pilot study we focused on three data quality dimensions: Completeness, accuracy and validity. These dimensions were measured for the following data : demographics (date of birth, gender, race, address, city, state, and zip code); laboratory results (plasma and urinary glucose, glucose POCT and HbA1C), and antidiabetic medications.
Results:
The patients in the study were consisting of more females than males (70% versus 30%) with more African American (57%) than white (43%). Validity: 100 % of the data was valid; all demographic data values are logic, and laboratory results are within the clinical reportable range. Completeness: Nearly 98.8% of the data was complete with only missing two plasma glucose and two HbA1c values. Accuracy: 100% concordance in all data elements.
Conclusions:
This study revealed that there is a complete concordance between data stored in AR-CDR and Epic data. The data in AR-CDR are complete and valid. Based on the results of this pilot study we confirm that the data warehouse has a high quality for clinical research.

B-029

Interface for the Bio-Rad D100 with Sunquest: Challenges and Solutions

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Background: Recently we purchased two Bio-Rad D100 instruments for hemoglobin A1c (HbA1c) analysis. The D100 instruments we acquired had internal HB Advisor software. The Advisor software screens the chromatograms against 32 programmed rules and flags samples that have parameters outside the limits of the stated rules. The flags were transmitted to Sunquest; however, the results were not held for review because they were considered by Sunquest as order flags and not as result flags. The discussions between our lab staff, our IT specialists, Bio Rad and Sunquest led to the

realization that the original Bio Rad interfaces do not lead to actions taken by the Advisor software when identifying the results with parameters outside the predefined limits of the rules. The aim of this work was to develop an interface allowing all results to be exported to Sunquest with non-flagged results to auto-release and flagged results to be held for review. **Method:** Various patient samples were ordered in the test environment of Sunquest. The patient samples were a mix of normal and abnormal HbA1c values, as well as some hemoglobin variant specimens. The results that were transmitted to Sunquest were manually verified for accuracy. A series of English Text Codes (ETC) were defined to be appended as appropriate to explain or assist clinicians to interpret the results, i.e., why there is no HbA1c value provided or to inform clinicians that a possible hemoglobin variant may be present to affect HbA1c results. **Results:** Initial interface allowed Sunquest to only receive non-flagged results. The second version of the interface had all results, flagged or not, transmitted to Sunquest. The third version auto-released all non-flagged results and held the flagged results in Sunquest for review. **Conclusion:** Collaboration between our lab staff, IT specialist, Bio-Rad, and Sunquest was critical to the successful improvement of interface to meet the clinical needs. The resulting interface allowed proper handling of HbA1c results with parameters outside of the predefined rules.

B-030

Impact of the Atellica Solution on User Hands-On Time

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Background: Daily maintenance, quality control, and reagent loading on analyzers consume a significant amount of technicians' time in a core laboratory. The Atellica® Solution automates daily maintenance and quality control, and provides on-the-fly reagent loading. This study assessed the impact of the Atellica Solution on operator hands-on time spent on these three activities, compared to the existing (legacy) instrumentation in our laboratories. **Methods:** Legacy instrumentation was chosen to match the Atellica Solution throughput as per the manufacturer's specifications. Equipment at Laboratory1:

- Atellica Solution: one Atellica® Sample Handler with two Atellica® CH 930 Analyzers and two Atellica® IM 1600 Analyzers; entire configuration connected to Aptio® Automation via a single connection.
- Legacy instrumentation: one ADVIA® 1800 Clinical Chemistry System, one ADVIA® 2400 Clinical Chemistry System, and four ADVIA Centaur® XPT Immunoassay Systems; each system connected to Aptio Automation via a separate connection.

Equipment at Laboratory 2:

- Atellica Solution: one Atellica Sample Handler with one Atellica CH 930 and one Atellica IM 1600 Analyzer; entire configuration connected to Aptio® Automation via a single connection.
- Legacy instrumentation: one ADVIA 1800 Clinical Chemistry System and two ADVIA Centaur XPT Immunoassay Systems; each system connected to Aptio Automation via a separate connection.

We then recreated a typical day in our laboratories during core working hours by using the workload usually processed by the legacy instruments listed above. 65% of the normal workload at Laboratory 1 was processed: 11,500 chemistry tests and 4250 immunoassay tests (15,750 in total) from 2700 tubes over 11 h. At Laboratory 2, 45% of the normal workload was processed: 6700 chemistry tests and 700 immunoassay tests (7400 tests in total) from 800 tubes over 8 hours. Tubes were batched and loaded on the automation system in 10 minute intervals to simulate arrival on a typical day. The test mix on each tube was also based on a typical day's mix in our laboratories. To achieve like-for-like comparison, in both laboratories this experiment was executed twice, with exactly the same tubes, timings, and test mix, but once with the legacy equipment and once with the Atellica Solution. Operator time spent on daily maintenance, reagent loading and quality control was recorded in both laboratories. **Results:** At Laboratory 1, time spent on daily interactions was 3 hours 50 minutes with the legacy instrumentation and 1 hour 2 minutes with the Atellica Solution. This represents a reduction of 73%. At Laboratory 2, time spent on daily interactions was 2 hours 41 minutes with the legacy instrumentation and 49 minutes with the Atellica Solution. This represents a reduction of 69%. **Conclusion:** The operator hands-on time as defined by daily maintenance, quality control management and reagent loading is considerably reduced on the Atellica Solution compared to our legacy instrumentation. This result was observed both at Laboratory 1 and Laboratory 2. Siemens Healthineers supported the study by providing systems, reagents, protocols and contributed to data analysis

B-031

Throughput Evaluation of the Atellica IM 1600 Analyzer with Varying Clinical Immunoassay Test Mix

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Background: Longer assay turnaround times (as for infectious disease assays) decrease immunoassay analyzer throughput. Labs often mitigate result reporting delays by dedicating analyzers for serology testing, resulting in staffing, capital cost, and space-usage inefficiencies. The Atellica® IM 1600 Analyzer was engineered with dual incubation rings, the outer ring initially for all assays and the inner ring for longer-dwelling assays. **Objective:** To evaluate the impact on throughput that a varying proportion of longer-dwelling assays can have, and to generate real-life throughput data relevant to a typical lab. **Methods:** Using our core lab's normal immunoassay work profile, we performed five runs of 300 tubes with a fixed number of 665 tests: 2.2 immunoassay tests per tube (our usual test-per-tube ratio). The tubes were of mixed tests and not all with the same profile in order to represent a real workload. We utilized 32 Atellica® IM assays. For each of the five runs, however, we adapted the proportion of long assays to range from 6% to 47% while keeping the number of tubes and tests constant. The longer-dwelling assays use both inner and outer incubation rings and range from 28 to 54 minutes; short assays use only the outer incubation ring and range from 10 to 14 minutes. **Results: Table 1.** System throughput with an increasing proportion of longer-dwelling assays

Proportion of long assays	Time to complete 300-tube run (all tubes sampled)	Tubes sampled during the first hour	Tests initiated in the first hour	Results reported per hour after 30 min[a]
6%	2 h 10 min	148	345	295
16%	2 h 21 min	142	346	280
27%	2 h 23 min	141	362	296
36%	2 h 26 min	138	369	308
47%	2 h 34 min	135	369	295

[a] This metric accounts for system throughput after initial workload buildup. **Conclusions:** The system throughput was not impeded by the increase in the proportion of long assays from 6% to 47% of the test mix. Siemens Healthineers supported the study by providing systems, reagents, protocols and contributed to data analysis

B-032

Performance Evaluation of Clinical Chemistry and Plasma Protein Assays on the Atellica CH 930 Analyzer Across Five Sites

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Background: Studies were performed at five different sites to assess the analytical performance of several clinical chemistry (CH) and plasma protein assays on the Atellica® CH 930 Analyzer. The assays tested were Alb, ALT, AST, Ca, Cl, Crea_2, GluH_3, K, Na, TBil, TP, Trig, UN_c, APO A1, APO B, B2M, CRP_2, hsCRP. Studies included precision verification, linearity and method comparison studies with Siemens Healthineers assay(s) (Site A: Dimension Vista® System, BN ProSpec®; Site B: ADVIA® 2400 system, Dimension Vista®; Sites C, D, and E: ADVIA® 1800 system; Site D: BN II®). **Methods:** Analytical performance studies were performed at five sites across Europe. Precision verification was performed according to EP15-A3, method comparison by EP09-A3, and linearity by EP06-A. Precision verification studies included three concentration levels; each level of QC materials was tested as one run per day with five replicates per run, for five days, yielding a total of 25 replicates per sample for each assay. Method comparison studies for select assays were performed using approximately 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges. The number of levels of linearity material (LGC Maine Standards) ranged up to six depending on the assay; for each assay, three replicates per sample level were assayed.

Results: The within-run CVs ranged from 0.0% to 5.3% and the total CVs ranged from 0.0% to 8.2% across all assays on the Atellica CH Analyzer using non-pooled data at this time. Precision results agreed with the manufacturer's claims. Linearity studies were performed for all assays. At each of the five sites, for those comparisons completed to date, method comparison Passing & Bablock and Deming regression results were comparable to the manufacturer's stated IFU claims. Initial method comparison studies were performed at three sites to date. Assays tested on the Atellica CH 930 Analyzer vs. ADVIA 2400/1800 demonstrated good agreement between these three sites for UN_c [Site B(2400): y=1.06x-1.39 mg/dL; Site E(2400): y=1.00x-0.99 mg/dL; Site C(1800): y=1.03x-1.23 mg/L], Crea_2 (Site B: y=1.02x-0.012 mg/dL; Site E: y=1.05x-0.01 mg/dL; Site C: y=1.04x-3.63 mmol/L). Method comparison was completed at two sites to date for AST (Site E: y=1.05x-1.73 U/L; Site C: y=1.04x-0.16 nmol/L), and ALT (Reggio: y=1.05x-1.73 U/L; Site C: y=1.00x+150 mmol/L). Comparison of Atellica CH 930 and Dimension Vista was performed for at least two sites to date for APO B (Site B: y=0.93x-1.13 mg/dL; Site E: y=0.97x-8.2 mg/dL). **Conclusions:** Overall, all assays tested on the Atellica CH 930 Analyzer across five sites in Europe demonstrated acceptable precision. Generally, the precision results were consistent with the manufacturer's claims. The method comparison studies completed to date between Atellica CH 930 Analyzer assays and other assay(s) from Siemens Healthineers demonstrated good agreement. *Siemens Healthineers supported the study by providing systems, reagents, and protocols, and contributed to data analysis.

B-033

Performance Evaluation of Immunoassays on the Atellica IM 1600 Analyzer Across Six Sites

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Background: Studies were performed at six different sites to assess the analytical performance of several immunoassays (IM) on the Atellica® IM 1600 Analyzer. The assays tested were Fer, VitD, PSA, eE2, ThCG, PRGE, TSTII, TSH3-UL. Studies included precision verification, linearity, and method comparison with Siemens Healthineers assay(s) (Site A: Dimension Vista® System; Sites B, C, D, E, and F: ADVIA Centaur® XP/XPT System). **Methods:** Analytical performance studies were performed six sites across Europe. Precision verification was performed according to EP15-A3, method comparison by EP09-A3, and linearity by EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days, yielding a total of 25 replicates per sample for each assay. Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges. The number of levels of linearity material ranged up to nine depending on the assay. For each assay, three replicates per sample level were assayed. **Results:** Precision results agreed with the manufacturer's claims. The within-run CVs ranged from 0.0% to 6.8% and the total CVs ranged from 0.5% to 14.6% across all assays on the Atellica IM Analyzer using non-pooled data at this time. At each of the six sites, for those comparisons completed to date, method comparison Passing & Bablock and Deming regression results were comparable to the manufacturer's stated IFU claims. Linearity studies were performed for all assays. Initial method comparison studies were performed at three to four sites to date. Assays tested on the Atellica IM Analyzer and ADVIA Centaur XP/XPT demonstrated good agreement between four sites for eE2 (Site E: y=0.92x-8.5 pg/mL; Site B: y=0.96x-1.03 pg/mL; Site F: y=1.10x-10.1 pg/mL; Site D: y=0.99x-34.3 pmol/L), three sites for PRGE: (Site D: 0.999x-0.47 nmol/L; Site E: 1.01+0.04 ng/mL; Site B: 1.19+0.36 ng/mL), PSA (Site E: y=0.93x-0.02 ng/mL; Site B: y=0.90x-0.06 ng/mL; Site F: y=0.99-0.01 ng/mL), ThCG (Site E: y=0.96x+2.8 mIU/mL; Site B: y=1.07+3.46 mIU/mL; Site F: y=1.02+1.73 mIU/mL), TSH3-UL (Site E: y=0.95x+0.14 µIU/mL; Site B: y=1.06-0.00 µIU/mL; Site F: y=1.02x-0.02 mIU/mL); and, Fer (Site C: 0.99-0.46 ng/mL; Site E: y=1.05x-1.09 ng/mL; Site B: 0.89x-0.59 ng/mL). **Conclusions:** Overall, all assays tested on the Atellica IM 1600 Analyzer demonstrated acceptable precision, and method comparison with the assay(s) from Siemens Healthineers. Generally, the precision results were consistent with manufacturer's claims.

* Siemens Healthineers supported the study by providing systems, reagents, and protocols, and contributed to data analysis.

B-034

Use of National EHR Data Warehouse to Identify Inappropriate HbA1c Orders for Sickle-Cell Patients

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Background: The glycated Hemoglobin (HbA1c) test is one of the most important diagnostic and prognostic strategies for monitoring diabetes. However, the clinical utility of this test is questionable for Sickle-cell disease patients. Sickle-cell disease is a common hematological disorder that affects millions of people worldwide. In these patients, who are homozygous for the hemoglobin variant gene, only the glycated form of the variant can be found which would have a shorter lifespan (10-20 days) compared to a normal erythrocyte (90-120 days). These patients may suffer from anemia, increasing red-blood cell turnover and requiring transfusions as treatment, which can adversely affect the assessment of HbA1c as a marker of glycemic-control. Therefore, HbA1c tests may not be reliable to independently diagnose or monitor diabetes in sickle-cell patients. Assessment of other glycemic biomarkers such as fructosamine can act as an alternative test for this population. While there have been local analyses, no prior national level analysis of this ordering practice has been performed. **Objective:** To evaluate the frequency of the inappropriate HbA1c test orders and the prevalence of fructosamine test orders as an alternative to HbA1c test for sickle-cell patients in Truman Medical Center (TMC), Kansas City, MO in comparison with sickle-cell patients from other national hospitals. **Methods:** We used a Practice-Based Evidence approach based on de-identified, HIPAA compliant, electronic health record (EHR) data in the Cerner Health Facts ™ (HF) data warehouse. TMC is a contributor to this national dataset. We evaluated the frequency of inappropriate orders of HbA1c tests by comparing the 526 sickle-cell patients in TMC with 37151 sickle-cell patients from 393 national hospitals in the data warehouse. The conditional probabilities estimated from the Generalized linear mixed model (GLIMMIX) was used to rank the TMC with other national hospitals based on the inappropriate order percentage of HbA1c Test for sickle-cell patients in a particular year while controlling for covariates such as the characteristics of the hospitals. The sickle-cell patient cohort was further analyzed for appropriate fructosamine encounters relative to HbA1c tests in TMC with other national hospitals. **Results:** TMC had a higher percentage (32%) of sickle-cell patients with HbA1c tests when compared to the national hospital cohort (11%). The results showed that TMC ranks in the bottom 25% quartile when compared to the other national hospitals with respect to inappropriate HbA1c orders. Interestingly, analyzing fructosamine encounters determined that TMC has ten-fold higher sickle-cell patients (11%) who had at least one fructosamine encounter when compared to the sickle-cell patients (1%) in the other 10 national hospitals which had fructosamine encounters. However, the majority of those patients (68%) in TMC had both fructosamine and HbA1c tests of which 27% of these patients had both the tests in the same encounter. **Conclusion:** These findings indicate that inappropriate HbA1c orders in sickle-cell patients is a potential quality concern in TMC which needs to be addressed with sustainable interventions so that overtreatment or under-treatment of the diabetic condition in sickle-cell patients are avoided.

B-035

Re-imagining protein electrophoresis interpretative workflow for the 21st century

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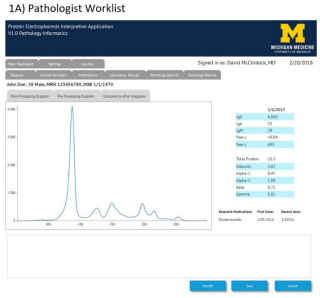
Background: Traditionally, the interpretative process for protein electrophoresis (PEP) has been time consuming and fraught with the potential for missing key information that could influence the result. Often, an elaborate paper-based workflow exists in order to get the relevant clinical and laboratory information to pathologists, with some working within three separate systems to finalize an interpretation. Fundamentally, a need exists to create a unified data "cockpit" that will allow integration of disparate data elements into a single user interface to aid PEP interpretation workflow. **Methods:** Our goal was to design a web-based application based on the following principles: 1) provide an automated, patient specific clinical data request from the EHR; 2) create a secure, flexible, and interactive user interface unifying relevant clini-

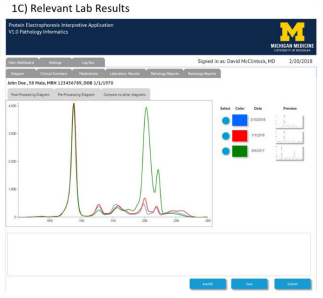
cal, laboratory, and PEP data; and 3) streamline the pathologist interpretation workflow to save both time and resources. Using recently available web services interface architecture, which enables pulling data from the EHR instead of just pushing data to it, we developed a custom web application integrating data from our EHR (Epic) and our protein electrophoresis vendor's middleware application (Phoresis, Sebia). **Results:** We successfully developed a working web services interface between the EHR and our web application that has the ability to pull patient specific clinical data from their medical record, including medication lists, laboratory results, clinical notes, and pathology and radiology reports. Additionally, we developed a way to import the native PEP data from the vendor middleware and render our own capillary electrophoresis and immunotyping diagrams within the web application. Figure 1 demonstrates four views from the web-application. **Conclusion:** The use of a modern web-based architecture to integrate multiple sources of disparate clinical data has great potential to revolutionize PEP workflows by consolidating multiple manual, labor intensive processes into a single streamlined application.

1A) Pathologist Worklist

1C) Relevant Lab Results



1B) Electropherogram with associated labs and medications



1D) Electropherograms, current and prior tracings

B-036

Performance evaluation of the UF-5000 automated urine analyzer

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Background: UF-5000 is a fully automated urine particle analyzer that is based on flow cytometry method. We evaluated the analytical performance of UF-5000. **Methods**: We assessed UF-5000 for precision, linearity and carryover rate using control materials. And a total of 268 urine samples were selected and analyzed by microscopic method and the two automation system, UF-5000 and UF-1000i. The results of analysis was compared between the automated analyzers with each other, and between UF-5000 and microscopic method. **Results**: Coefficients of variation (CVs) of control materials for the within-run precision of red blood Cells (RBC), white blood cells (WBC) and bacteria count were 3.13-9.32%, 2.41-8.56% and 3.74-6.55%, respectively, which were concordant with those announced by the supplier. And the CVs of epithelial cells and cast were 14.27-16.59% and 16.66-21.81%, respectively. In aspect of linearity, the correlation coefficient values of analytes were over 0.99. The carryover rate was less than 0.01% for RBC and WBC. And that of bacteria were 0.02%. The agreement rates within same grade between the UF-5000 and UF-1000i for RBC, WBC, epithelial cells, cast and bacteria were 88.8%, 92.2%, 89.6%, 90.7% and 79.9% respectively. The agreement rates within same grade between the UF-5000 and manual microscopy for RBC, WBC, epithelial cells, cast and bacteria were 77.5%, 77.5%, 76.3%, 87.5% and 68.8%, respectively. The agreement rates within one grade between the UF-5000 and UF-1000i, and between the UF-5000 and manual microscopy for all analytes were nearly 100%, respectively. **Conclusions**: UF-5000 showed a reliable analytical performance and good concordance with manual microscopic method.

B-037

Method Validation of Pleural and Ascites Fluids cell counts on Sysmex XN-1000 automated haematology analyser

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Background: Body fluid (BF) cell counts of white blood cells (WBC) and red blood cells (RBC) are of great diagnostic value where it aids in the diagnosis and treatment decisions of pathological conditions such as haemorrhages and inflammations. The estimation of cell counts using a haemocytometer, remains a challenge in clinical laboratories. This manual method has been considered the "gold standard" for decades, especially in fluids with low cell counts. It is often laborious, subject to high inter-operator variability and low reproducibility. The XN-1000 automated haematology analyser (Sysmex, Kobe, Japan) performs BF cell counting by using a dedicated BF mode. The aim of this study was to determine the analytical performance of the XN-1000 for counting cells in pleural and ascites fluids in the clinical laboratory of Khoo Teck Puat Hospital, a 720-bedded acute care hospital situated in the north of Singapore. **Methods:** We analysed 97 fluid samples (pleural, n = 32; ascites, n = 65) using the XN-1000 and compared the results with those obtained by the reference method. Samples with cell counts distributed over the analytical measurement range were used for correlation studies for RBC and Total Cell - Body Fluid (TC-BF). The fluids were collected in sterile containers according to established practises, then mixed till homogenous. Three millilitres was transferred into K2 EDTA vacutainers. These were then analysed for BF-TC and RBC on the XN-1000, using the open mode. While concurrently, 10 microlitres of sample was used to charge the haemocytometer to count and estimate the total nucleated cells and RBCs. **Results:** Imprecision studies yielded a coefficient of variation (cv) as follows, RBC: 2.8 and 3.7 (cell count = $0.026x10^6$/uL), 2.3 and 2.27 (cell count = $0.076 x10^6$/uL), TC-BF: 6.6 and 6.6 (cell count = $0.081x10^3$/uL), 3.1 and 3.14 (cell count = $0.313x10^3$/uL).Linearity study using serially diluted samples yielded the following findings: RBC: $R^2 = 0.9991$ (pleural) and $R^2 = 0.9992$ (ascites); TC-BF: $R^2 = 0.9963$ (pleural) and $R^2 = 0.993$ (ascites). Carryover studies show that there was no significant carryover interference between samples for RBC measurements, which was within the manufacturer's claim of 0.3 %. However, some interference is suggested for TC-BF. 26% increase in cell count could be observed in low cell count samples analysed immediately after a high cell count sample of TC-BF = $9x10^3$/uL. In the correlation study, both RBC and TC-BF demonstrated good correlation with the manual counting method: $R^2 = 0.994$ with slope of 1.14 and $R^2 = 0.986$ with slope of 1.00 respectively. **Conclusion:** Our evaluation data showed that the analytical performance of cavity fluids cell count on XN-1000 showed good agreement with the manual method.There is some evidence to suggest that preventive measures such as auto-rinse and background check should be carried out after analysing samples that have TC-BF count >$10x10^3$/uL. On average, an operator requires 15 minutes to count in duplicates by haemocytometer. The workflow is reduced to 2 minutes by automation. The incorporation of cell counting on XN-1000 eliminates the inter-operator variability and increases the productivity in our laboratory.

B-038

Abnormal Proportion-based Reference Intervals

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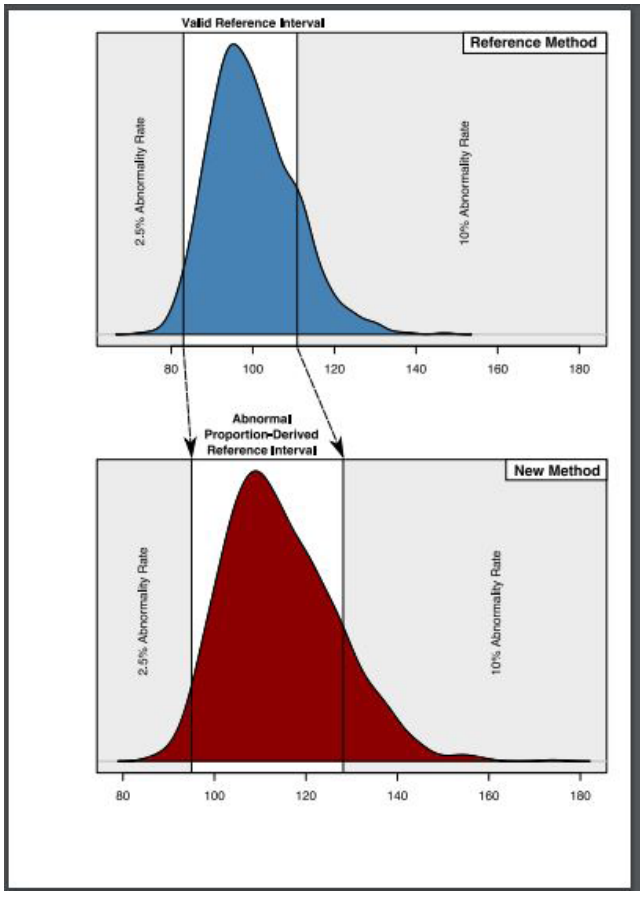
Background: While accurate reference intervals are essential for interpretation of laboratory results, they are difficult and expensive to establish and maintain. Under stable conditions, each laboratory has a consistent abnormality rate over time. Given this stable rate, we hypothesized that reference intervals could be back-calculated from the abnormality rate. The objective of this study was to develop a method to derive reference intervals from patient data using the proportion of abnormal results. **Methods**: To test the abnormal proportion-based interval hypothesis, we used patient data from a large chemistry analyzer replacement project (from Beckman Lx20 to Siemens Vista). This project included generation of reference intervals from healthy volunteers (n=200) using non-parametric methods according to CLSI guidelines. Abnormal proportion-based intervals were calculated from existing abnormality rates for common chemistry tests (proteins, electrolytes, and enzymes) using >=1000 sequential patient results. Abnormal proportion-based intervals were calculated by determining the cutoffs on the new instrument (Vista), which best matched the previous abnormality rate (Lx20); various patient sample sizes for the new instrument were tested (n=20-10,000). Proportion-derived intervals were compared against the gold-standard healthy volunteer-derived reference intervals. Accuracy was calculated as the percent difference between the gold standard reference

interval and the derived interval. This approach was also evaluated using simulated data with different distribution shapes using the statistical programming language R. **Results**: In both real-world patient data and simulations, abnormal proportion-derived reference intervals were highly accurate (>98% similar to true values). Proportion-based intervals were robust and highly accurate for any distribution of patient results. Even very small patient sample sizes of n=20 yielded 90% accuracy; larger patient samples (>500) yielded accuracy rates of >98%. **Conclusion**: Abnormal-proportions can be used to effectively transfer reference intervals between instruments within the same laboratory. Advantages of this method include application to any patient data, no data distribution assumptions, and robustness to sample size.



B-039

Automated Laboratory-based Population Health System for Hepatitis C Birth Cohort Screening

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Background: . Hepatitis C virus (HCV) is the most common blood borne pathogen in the U.S. and leading cause of liver failure, hepatocellular carcinoma, and liver transplants. Due to highly effective anti-viral medications, most patients can be cured. As a result, identifying patients with occult HCV infections has emerged as one of the most important challenges in combating this disease. In 2012, the CDC recommended universal screening of individuals born between 1945-1965 (birth cohort) due to higher risk of HCV infection. Despite campaigns to make healthcare providers and the general population aware of HCV screening guidelines, many chronic infections remain undiagnosed. The objective of this project was to evaluate the effectiveness of an automated laboratory-based population health system in collaboration with healthcare providers to improve HCV birth cohort screening. **Methods:** A birth cohort registry of active patients seen within 2 years and without prior testing for anti-HCV was maintained at the Tucson VA Medical Center. With medical staff approval, the registry was used to generate and mail automated letters to targeted patients. The letter described the purpose and recommendations for HCV screening and served as a test requisition on behalf of patient's healthcare provider. The letter provided contact information to a designated nurse for questions and encouraged patients, if needed, to discuss testing with their healthcare provider. Upon completion of testing, another letter was automatically sent to the patient with results, if negative. Patients with positive results for antibody and HCV RNA, were notified of referral to specialist for evaluation and management. **Results:** Between October 2015 and January 2018, 9186 patients received letters. A second letter was sent to subgroup of 1732 (18.9%) who remained untested after one year. Overall, 4605 (50.1%) who received letters were tested. Of these, 2150 (46.7%) orders were from patient letter requisitions while the remaining 2455 (53.3%) were orders placed by healthcare providers. Among 7454 patients receiving a single letter, 1879 (25.2%) and 2180 (29.2%) were tested by letter requisition and healthcare provider orders respectively. Among 1732 patients receiving a second letter 271 (15.6%) and 275 (15.9%) were tested by letter requisition and healthcare provider orders respectively. The average annual anti-HCV test volume (n=4051) during letter program was about twice that of the prior 3-year average (n=2175). A total of 120 (2.6%) screened patients were positive for anti-HCV of which 46 (1.0%) were positive for HCV RNA. The nurse received an average of one phone call per day and most questions were administrative (e.g. access to testing, appointments) rather than clinical. **Conclusion:** These results show that an automated laboratory-based population health screening program was effective for increasing the detection of occult HCV infections. Repeat notification of untested patients was less effective. In addition to population health screening, a similar strategy using registries for automated notifications and results reporting might likewise provide value-based collaborative support to healthcare providers to assist with managing patients who, for example, require periodic laboratory monitoring for chronic disease (e.g. diabetes), high-risk medications (e.g. oral anticoagulation) or post-treatment surveillance (e.g. tumor markers).

B-040

Statistical Software for QC/QA. A Practical Example with Minitab® for Short-term and Long-term Precision Evaluation with two cobas® c501 Instruments.

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Background. Shewhart's single observations chart (L. J. chart) and Westgard's rules were proposed for precision evaluation of methods in laboratories without statistical software. With the advent of the LIS both became available for electronic, real time quality control (QC) practices. We present two practical examples for the use of statistical software for QC, exploratory, inferential and capability data analysis. **Materials.**Two cobas® c501 instruments (Roche). Analytes: glucose (reagent lot #28132, exp. 1/31/2019, Roche); calcium (reagent lot #300983, exp. 2/27/2019, Roche). QC material (level 1, lot 31841, exp. 9/30/2018; level 2, lot 31842, exp. 9/30/2018, Bio-Rad), Unity Real Time® (Bio-Rad), Minitab® (version 17, Minitab Inc.) statistical software. Short-term precision study: five repeated assays with each control material throughout 24 hours for seven consecutive days. Long-term precision study: one assay with each control material every 8 hours for 180 consecutive days. The data were electronically transferred to Minitab and analyzed with descriptive, exploratory, inferential, QC and capability statistical techniques. **Results.** For the short-term precision study the General Linear Model (GLM) and the parallel box plots for both glucose and calcium showed that for both levels of QC material there were no statistically significant differences (P>0.05) between days and instruments. The data had a quasi-normal distribution (normal probability plot), independence (autocorrelation tests, T<1.5), there were no statistically significant differences between variances by days and instruments (P>0.05) and no parallelism was displayed by the Otelling'sT^2 graphic representation. Furthermore, the L.J. chart showed that the upper and lower control limits (mean-3s and mean+3s), as calculated with standard deviation estimated with the GLM and the appropriate correction factor, were smaller than the upper and lower specification limits as calculated with the CLIA's criterion for acceptable total error (Glucose: target value +/- 6 mg/dL, +/-10%, greater; Calcium: target value +/- 1 mg/dL). The long-term precision at 180 days showed that fluctuations of the mean were associated with new shipments of reagents. These means fluctuations were detected early with the Cumsum chart. While for glucose the means of Level 1 and 2 fluctuated within +/-1s, for calcium the means fluctuations exceeded +/-1s. These patterns could be easily demonstrated with the Shewahrt's mean chart, lowess, parallel box plots. The graphic display of Otelling's T^2 showed borderline parallelism. However, since the mean fluctuations were not associated with differences in patient specimen values (difference between reagent shipments <<0.5 mg/dL, with a total allowable error of 1mg/dL), the control mean could be adjusted so that daily QC practices were not affected by type I error. The capability analysis at six months showed for glucose level1: Cp=2.6, Cpk=2.6, level2: Cp=2.8, Cpk=2.7; for calcium level1: Cp=2.6 , Cpk=2.5, level2 Cp=1.7, Cpk=1.6. These results indicated that the methods performance was

acceptable. **Conclusions:** The ability to capture electronically QC data in real time and subsequent transfer to Minitab allowed for deep critical evaluation and immediate detection of unnatural patterns. Statistical analysis of long-term precision data with appropriate graphic displays allowed an immediate, deeper understanding of the sources and dynamics of methods variation. Furthermore, capability analysis techniques evaluated the method performance.

B-041

A non-parametric quantile regression method to establish continuous reference intervals

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Background: Reference intervals are critical for laboratory test interpretation. For several analytes, reference values exhibit dynamic trends in relation to age, especially throughout childhood and adolescence. Therefore, reference intervals have traditionally been partitioned by age, often arbitrarily chosen as evenly spaced age groups or confirmed by less than optimal statistical methods. To accurately represent the dynamic relationship of age and analyte concentration and improve the accuracy of laboratory test interpretation, continuous reference intervals should be used. As there are numerous methods that can be used to estimate continuous reference intervals, we explore nonparametric quantile regression methods, which offer several advantages, and recommend a method for use in the field of laboratory medicine. **Methods:** Data from the CALIPER project were used to apply and explore different nonparametric quantile regression methods. We focused on two analytes, total bilirubin and alkaline phosphatase, from healthy pediatric subjects 1-<19 years of age (samples from subjects <1 year were excluded). Three different nonparametric quantile methods were used to estimate the 2.5th and 97.5th quantile curves as a smooth function of age, including local polynomial quantile regression, quantile regression with restricted cubic splines and quantile regression via B-splines with different penalties and monotonicity and non-crossing constraints. **Results:** Nonparametric quantile regression methods are robust to various departures from assumptions such as normality, symmetry, linearity, and variance homogeneity. These methods are also not sensitive to outliers and are thus very flexible and powerful methods to estimate continuous reference intervals. The reference curves for total bilirubin and alkaline phosphatase established using all three methods (i.e. local polynomial quantile regression, quantile regression with restricted cubic splines and quantile regression via B-splines with different penalties and monotonicity and non-crossing constraints) were compared. The results revealed that the quantile regression via B-splines with different penalties and monotonicity and non-crossing constraints is the most flexible method to model the nonlinear relationship between age and analyte concentration. In addition to generating smoother reference curves, the non-crossing constraint property of this method allows us to estimate the reference curves with no crossing point, while its monotonicity constraint property enables us to choose various flexibility fits (i.e. non-increasing, unconstrained, or non-decreasing). **Conclusion:** We recommend using the nonparametric quantile regression via B-splines with different penalties and monotonicity and non-crossing constraints to develop continuous reference intervals. This method uses cross validation to choose the optimal value of the smooth parameter, thus removing subjective estimations. Besides being very practical, another advantage of this method is its ability to accurately model nonlinear patterns with respect to age. In addition, due to non-crossing and monotonicity restrictions, the method produces more biologically plausible estimates. Lastly, we provide information about the methods' corresponding functions in R software to aid other laboratories to apply these methods to other databases of healthy subjects.

B-042

Utility of reactive urine strip in diabetes diagnostic screening

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Background: The glucose that is filtered by the glomeruli is almost completely reabsorbed in the proximal tubules. The presence of glucose in urine (glycosuria) is an anormal finding that usually occurs when the blood glucose concentration exceeds the renal threshold (180 mg / dl), it would indicate a possible diabetes. The aim of this study is to analyze the validity of a semiquantitative method of glycosuria screening. **Methods:** The urine of 332 patients was analyzed. The glucose in the first urine isolated in the morning was determined by two different methods: - Semi-quantitative: colorimetric method based on an enzymatic reaction (glucose oxidation), H13 urine strip, DIRUI H-800 PLUS (RAL®). The concentration of glucose is directly proportional to the color developed in the pad. The instrument measures the color change of the test strip on a scale of 100 to 1000 mg / dL. - Quantitative: glycosuria was measured by the reaction to the end point in the COBAS C311 (ROCHE DIAGNOSTIC®). The statistical analysis was carried out by determining the area under the curve (ROC). **Results:** The minimum value obtained was 0.1 mg / dL and the maximum value 2268.0 mg / dL. The average obtained was 225.74, median 5.85; standard deviation 633.13; interquartile range = 8.15. The Mann Whitney test between both methods did not show statistically significant differences ($p < 0.0001$). Glucose is positive in the test strip when the urine has a concentration higher than 46.9 mg / dL of glucose, with a sensitivity of 95% and a specificity of 100%, with an area under the curve of diagnostic accuracy = 0.953 ($p < 0.0001$). **Conclusion:** All the urines with a glucose concentration higher than 46.9 mg / dL were positive, so the test strip detects glucose values above this concentration. Despite its low sensitivity, the reactive strip is considered a good screening method for the detection of glucosuria in diabetic patients, due to its low cost and its methodological simplicity. A positive glucosuria would lead to the determination of serum glucose (which becomes more relevant in patients where blood extraction is difficult). We must warn that the interpretation of glucose levels in urine should always be validated in parallel to blood glucose levels. Given the constant increase in the prevalence of diabetes (due to the age profile of the population in our environment) and the underdiagnosis of type 2 diabetes, we observed an increase in late complications and associated pathologies. Detection through routine screening of diabetes, in Primary Care, by a semi-quantitative method such as the urine dipstick, contributes significantly to improve the quality of patient's life diabetic patient, by means of the initiation of an early treatment during the phase pre-diabetes and to reduce health spending.

B-043

Evaluation of diagnostic testing workflow between routine manual Laboratory-Developed Tests (LDTs) and an automated LDT performed on the cobas® 6800 System

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Background: Lab-Developed Tests (LDTs) serve a critical role in patient management within clinical laboratories for the detection of pathogens when a FDA-approved IVD option is unavailable. Unfortunately, LDTs are typically labor intensive and often require multiple work areas and instruments to complete the testing process and to maintain a uni-directional workflow. Additionally, multiple rounds of manual specimen identification and confirmation are required to maintain traceability within the clinical setting, further increasing the complexity of the laboratory workflow. Adoption of automated solutions may mitigate some of these burdens by providing a sample-in-result-out workflow, including multi-level specimen tracking and traceability. The **cobas**® 6800 System is a fully automated, sample-to-result system for routine or high-volume molecular testing that relies on real-time PCR detection; the open channel functionality (**cobas** omni Utility Channel) supports the development and implementation of user-defined PCR tests using TaqMan® technology.The objective of this study was to comparatively assess the workflow and timings for three routine manual LDTs, performed within clinical laboratories, with a qualitative LDT performed on the **cobas** 6800 System. **Methods:** Four LDTs in three independent laboratories, employing unique extraction and amplification units including one LDT performed on the automated **cobas** 6800 System, were observed. The total time and number of interventions/steps were captured for each activity within the pre-analytic, analytic, and post-analytic phases of the LDT process. All observations were by invitation of the clinical laboratories and of routine manual LDTs performed by trained and experienced personnel assigned to the particular assay. **Results:** Each of the three manual LDTs required ten or more interventions with a combined average of 70 steps to complete the testing process including 7-8 unique dedicated work areas throughout the laboratory, which consisted of 2-3 hoods, 2 instruments, and 2-3 computers. With optimized run sizes, the average active hands-on time for each of the three manual LDTs was over 78 minutes and total true walk-away time averaged approximately 150 minutes among the tests. Automation resulted in an overall reduction in the number of interventions and steps to 3 and 13, respectively, reduced the dependence on multiple work areas (utilizing only 2); the hands-on time was reduced by 91%, down to 7 minutes, while the true walk-away time was increased to approximately 192.5 minutes (an increase of over 28%). **Conclusion:** Through the automation process, many manual steps of traditional LDTs, including pipetting and reagent preparation, as well as the need for multi-step verifications, are eliminated. The sample-in-result-out workflow on the **cobas** 6800 System results in reduction in overall hands-on time, the dependence on multiple instruments

and work areas, while also maximizing walk-away time. On-board specimen tracking and validity checks on the automated system also allow for improved traceability and ultimately, minimize the risk of user or clerical errors.

B-044

Automating a MALDI-TOF Mass Spectrometry Replacement of Gel Electrophoresis in the Clinical Laboratory

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Background: Our high throughput clinical electrophoresis laboratory has stagnated in tests per full time employee (FTE) as manual, semi-batch runs and paper result tracking are limiting our gel-based detection of monoclonal immunoglobulins (M-proteins). We recently described a MALDI-TOF MS method (MASS-FIX) as a plausible replacement for gel-based immunofixation (IFE). However, to utilize MASS-FIX, a fully validated automated method meeting current analytical capabilities was required. The objective of this study was to clinically validate an automated version of our MASS-FIX assay suitable for replacing IFE in our high throughput clinical laboratory. Our aim was to automate pre-analytical sample processing, improve positive specimen identification, improve ergonomics by reducing manual pipetting, reduce paper data storage and improve FTE utilization without impacting turnaround time (TAT). **Methods:** Serum samples were processed in batches of 32 or 64 and loaded onto a liquid handler (Starlet, Hamilton Robotics) along with reagents and a barcoded 384 well plate. The pre-analytical steps included: 1. Pipetting immunopurification beads specific for IgG, IgA, IgM, kappa and lambda immunoglobulins (CaptureSelect, ThermoScientific) to unique wells, 2. Adding 10 mcl of patient serum, 3. Removal of nonspecific proteins by washing, 4. Eluting the purified immunoglobulin 5. Reducing the sample to separate the heavy and light chains. The resulting 384 plate was transferred to a second liquid handler designed for low-volume pipetting (Mosquito HTS, ttpLabtech) for MALDI plate spotting. MALDI-TOF mass spectra were collected using a Microflex LT (Bruker Daltonics). An integrated in-house developed software was utilized for data analysis, history tracking, and result reporting. Residual serum samples (N=1043) were run using the automated system and results were compared to prior IFE results. **Results:** The automated MASS-FIX method was capable of meeting the validation requirements of accuracy, limit of detection, sample stability and reproducibility with a low repeat rate (1.5%). Out of 1,043 samples, 338 were positive by IFE and 705 had no M-protein detectable by IFE. The overall qualitative concordance was 95.8% for IFE positive samples and 95.8% for IFE negative samples. The limit of detection of MASS-FIX was similar to IFE at 15-60 mg/dL, depending on the patient's immunoglobulin background level. The automation and integrated software allowed a single user to process 320 samples in an 8 hour shift. Software display allowed for rapid and easy identification of M-proteins. Additionally, the entire system maintains positive sample identification, greatly reduces manual pipetting and does not significantly impact TAT. The additional benefit of electronic record keeping is to regain at least 100 square feet of lab space used for paper patient history files. **Conclusion:** MASS-FIX is ready for implementation in a high-throughput clinical laboratory. In addition to the analytical improvements, the major advantages of this method over our current gel-based assay include automation, electronic record keeping and positive sample identification.

B-045

Data Mining and Visualization for Monitoring Suspected Healthcare-Associate Infection

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Background: Healthcare-associated infection (HAI) control is one of the most important work in hospital. Early outbreak detection prevent patients from getting worse disease condition and also save a lot of medical expenses. The surveillance and timely feedback of HAI-related risk assessments can be complicated, especially for a 3,000 beds hospital. The object of this study was to build a data visualization system for monitoring suspected HAI with ease. **Methods**: Database was used to store bacterial species, warning threshold, daily reports and count for every microorganism. The baseline and warning threshold for every bacteria was calculated from September 2013 to March 2015. The total, positive and daily average report count were 260,779, 66,446 and 118 respectively. Warning threshold for every bacteria was defined as the mean of daily culture report count plus 1.28 standard deviation(SD). Scheduled computer program collect daily culture report and compare with warning threshold. Once the report count is higher than the warning threshold, the program will send an email to notify infection control staff. If there were 3 and more patients has same microorganism report in the same day, the system will fire a warning signal, too. Data visualization was presented in web format with Google chart application. It include bacterial daily culture count trend, specimen distribution, and ward distribution. **Results**: The daily report count mean±SD and warning threshold for 3 of most common bacteria were *Escherichia coli* (18.5±5.7, 26), *Pseudomonas aeruginosa* (11.1±4.7, 17), *Klebsiella pneumoniae* (9.0±4.0, 14). During the system test, the warning times and rate for 3 bacteria in 61 days were 8/61, 13.1%; 3/61, 4.9% and 4/61, 6.6% respectively. The average number out of 68 ward for 3 bacteria in a month that happened an event of 3 and more patients has same microorganism report in the same day were 6.7, 5.9 and 2.6 respectively. With a retrospective study of a HAI event of normal saline contamination which was caused by *Ralstonia pickettii.* The system fired warning signal at the event start. **Conclusions**: This monitoring system provides automated data collection and summarization. It also provides data visualization as a chart view, which makes the management and prevention of the infection control more conveniently and efficiently.

Wednesday, August 1, 2018

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

Infectious Disease

B-046

Prevalence of Hepatitis B Surface Antigen Among Apparently Healthy Individuals in Ibadan Nigeria

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Background
Hepatitis B Virus (HBV) screen-and-treat programme targeting the general population is a pragmatic public health intervention. Preventive strategies are reasonable options in resource-limited countries. This study was aimed at evaluating the prevalence of HBV in Ibadan a cosmopolitan city, and to provide baseline data for onward intervention and treatment.
Methods
The study was cross-sectional. A total of 178 participants (118 females and 60 males) apparently healthy aged 10-60 years participated in the study. Socio-Demographic characteristics and risk factors for HBV infection were documented in a pretest structured questionnaire. Blood samples were obtained for qualitative detection of HBsAg using rapid chromatographic immunoassays with test kits from Micro Point (China) having sensitivity, specificity and accuracy of >99%, 97% and 98.8% respectively. Data was analyzed using Chi square.
Results
Prevalence of 6.2% was observed in the study population. HBV positivity of 4% was observed among the participants in the age range 41-50years and 0.6% among adolescent 11-20years. A prevalence of 3.4% and 2.8% were reported for females and males respectively.
Conclusions
High prevalence of asymptomatic HBV infection was observed among the adult population. Female gender had a higher spread of HBV and may have negative impact on the younger population if not treated because they are care givers. Population target screening, treatment and public health enlightment will benefit the population and also reduce the socioeconomic implication of HBV
Key Words: Asymptomatic Hepatitis B Virus, Prevalence, Ibadan

B-047

Development of a Bartonella henselae specific Human IgG ELISA

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1. Introduction *Bartonella henselae* causes cat scratch disease (CSD), an often self-limiting lymphadenitis in immunocompetent patients, and several other clinical entities. While cats are the natural reservoir for *B. henselae,* the pathogen is transmitted by cats, cat fleas and eventually by other arthropods. The clinical symptoms underlying CSD might be similar to those being suspicious for malignant tumors. Thus, an easy and reliable test for *B. henselae* infections is highly desirable. **2. Objective** The aim of this study is to design an ELISA for detection of *B. henselae* to improve the shortcomings of the currently used immunofluorescent test (IFT), e.g., objective and reproducible results and less hands-on time. **3. Material and Methods** Test development is based on different *B. henselae* strains and quality assured patient sera [(a)sera positively tested for anti *B. henselae* antibodies via IFT, (b) patients with typical symptoms, (c) sera of patients with PCR-based infection diagnosis]. Antigens were separated by ion exchange chromatography and fractions examined in lineblots. Potential fractions were further tested and optimized for ELISA. **4. Results** Patients with *B. henselae* infections show different patterns of antibody expression in western blots. Thus, there is obviously no universally usable antigen for diagnosis detectable. Crude antigen preparations (liquid grown or with cell culture) are not working reliably as they do not react with numerous patient sera. However, our tests show that there are certain protein fractions from *B. henselae* which react reliably and results from lineblots were successfully transferred to an ELISA-format with sufficient sensitivity. **5. Conclusion** We show a strategy for antigen testing and selection from *B. henselae* protein preparations for ELISA-based serology. Further processing of antigens is under investigation so that in future an ELISA for *B. henselae* is possible.
Funding This study is financed by the state Hesse within the LOEWE III project.

B-048

Evaluation of individual and combined markers of urine dipstick parameters and total lymphocyte count as a substitute for CD4 count among HIV infected patients in resource-limited communities in Ghana

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Background: The diagnosis of HIV infections is based on CD4 count. However, most developing countries lack availability of CD4 count machines, reagents and expertise. The need to develop a less expensive and readily available diagnostic approach is warranted. We evaluated the individual and combined levels of urine dipstick findings and total lymphocyte count (TLC) as surrogate markers for CD4 count in a low-resourced community in Ghana. **Methods:** This cross-sectional study recruited 200 HIV infected patients from the Saint Francis Xavier Hospital, Assin Fosu, Ghana. CD4 counts, complete blood count (CBC) and dipstick urinalysis were measured for all participants. The threshold values were determined as <350 cells/µl for CD4 (new WHO criteria for starting HAART), <1200 cells/µl for TLC and ≥+ on urine dipstick analysis. CD4 T lymphocytes count was determined using the Becton Dickinson (BD) FASCount system, CBC was analysed using a five (5)-part automated blood analyzer (HORIBA Yumizen H500, Japan) and urinalysis was performed using dipstick urinalysis strips (Accu-Tell, ABT-UM-A33). Other signs of active infections and conditions that may interfere with urine dipstick analysis were also excluded. Receivers operating characteristic (ROC) curve was performed on the markers to obtain sensitivity, specificity, area under the curve (AUC), positive predicted values (PPV) and negative predicted values (NPV) were performed. **Results:** The mean age of participants was 43.09years. Proteinuria≥+ [(aOR=4.30(3.0 to 18.5)], leukoctyuria ≥+ [aOR=2.91(1.33 to 12.5)], hematuria ≥+ [(aOR=2.30(1.08 to 9.64)] and TLC <1200 cells/µl [aOR=3.26(3.94 to 15.29)] were significantly associated with increased risk of CD4 count <350 cells/µl respectively. Using the individual markers, the best substitute marker for predicting CD4 count <350 cells/µl was proteinuria at a cut-off point ≥2++, AUC of 0.973, sensitivity of 97.6%, specificity of 100.0%, PPV of 100.0% and NPV of 89.1%. A combination of ≤1200 TLC + ≥2++ (leukoctyuria + proteinuria + hematuria) yielded an AUC of 0.980, sensitivity (72.8%), specificity (100.0%), PPV (100.0%) and NPV (97.9%). **Conclusion:** Proteinuria could serve as an early non-invasive screening tool for identifying HIV infected individual, but the combination of proteinuria, leukocyturia, hematuria and TLC serves as a better substitute marker for CD4 count in monitoring the disease progression among HIV patients in resource-limited communities.

B-049

Cost Savings from Appropriate Utilization of Procalcitonin (PCT) in an Acute Hospital

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Background: Procalcitonin has clinical utility in the initial diagnosis and subsequent management of patients with sepsis, particularly in guiding antibiotic therapy. Inappropriate utilization of this test adds unnecessary costs to patients without a concomitant improvement in patient outcomes. Khoo Teck Puat Hospital is a 700-bedded acute hospital with an Emergency Department and Intensive Care Units. **Objective:** We describe our laboratory's experience in facilitating appropriate usage for procalcitonin and measuring cost savings. **Methods:** As part of a resource utilization review, we tracked the number of procalcitonin requests in our hospital over a four-year period from January 2014 to December 2017 using the Laboratory Information System (LIS). The data was analysed, and feedback given to high usage wards such as surgical intensive care units. Topics on resource utilization and the role of procalcitonin were also raised during clinical-pathological conferences, departmental meetings, with active involvement of infectious disease physicians and anti-microbial stewardship pharmacists. **Results:** From 2014 to 2017, a total of 69,101 procalcitonin results were reported. The number of procalcitonin requests fell from 22,122 in 2014 to 12,533 in 2017 (43% reduction). The was a 14% to 19% year-on-year reduction in the total number of procalcitonin requests. The mean number of requests per patient-visit

fell from 1.76 to 1.68, 1.53, 1.44 from 2014 to 2017 respectively. The number of emergency department consults and admission numbers remained constant during this period, suggesting the fall was attributable to more judicious use in procalcitonin, rather than a drop in patient load. The number of patients who had a large number of procalcitonin requests also decreased. In 2014, there were 7 visits with more than 30 procalcitonins ordered. In 2017, no visits exceeded 30 procalcitonins. The highest number of procalcitonin ordered in a single visit was 28 in 2017. Overall, the percentage of visits with more than 10 procalcitonin decreased over the years and were 1.05%, 1.02%, 0.73% and 0.56% for 2014, 2015, 2016 and 2017 respectively ($p<0.001$). This represents a reduction of 700 procalcitonin requests per year among high usage patients, translating to a cost savings of SGD 52,500 per year. Among visits with procalcitonin requests, the percentage of patients who had more than one procalcitonin decreased from 30% to 25%, 23% and 20% from 2014 to 2015, 2016, 2017 respectively. Among patients who had multiple sets, the mean retesting interval appropriately increased from 2.84 to 2.83, 2.99 and 3.28 days from 2014 to 2017 respectively. **Conclusion:** While the repertoire and costs of laboratory tests continue to increase, our review shows that the clinical laboratory plays a key role in resource utilization. Active surveillance, collaboration with clinicians, providing regular and objective feedback to physicians, may alter ordering behavior and contribute to cost effective care.

B-050

PREVALENCE OF HEPATITIS B VIRAL INFECTION IN APPARENTLY HEALTHY ADULT PATIENTS OF PUBLIC PRIVATE PARTNERSHIP LABORATORY, UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA.

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Background: Hepatitis B viral infection is a chronic infection which could lead to chronic liver disease and in turn eventually leads to several other clinical outcomes associated with chronic liver disease. This study was carried out to determine the prevalence of hepatitis B viral infection within a period of eight month at the laboratory with the highest number of clients in the University college Hospital, South western part of Nigeria. **Materials and Methods:** The laboratory records of hepatitis B viral infection in apparently healthy adults, from July 2016 to March 2017 of our laboratory was compiled. Hepatitis B surface antigen (HBsAg) was assayed using electrochemiluminescence immunoassay "ÉCLIA" method on Cobas E immunoassay analyser, making use of sandwish test principle. Levels 1 and 2 quality control material specific for HBsAg produced by Roche was always included in our daily work. Any result less than 0.9 Col was considered non reactive while results between 0.9 to 1.0 were considered borderline results which were repeated for confirmation. Any result greater than 1.0 was considered reactive. **Results:** A total number of 503 apparently healthy adult patients were investigated. 288 (57.3%) of the population were male while 215 (42.7%) of the population were female. A total of 108 (21.5%) of the overall population tested positive to HBsAg. Of the 21.5%, 15.3% were male while the remaining 6.2% were female. This suggested a higher prevalence of HBsAg in men than in women. **Conclusion:** A decline in the level of hepatitis B virus infection could be achieved through public enlightenment campaign, massive immunization of children and adults who are at risk. Effective diagnosis, treatment and follow-up should be provided for those already infected.

B-051

Incidence of reactive HIV results during 2017 in private lab and oficials statistics

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Background: Since the 80s, Brazil has instituted as state politic, a Public Healthcare System that, in essence, values the universal, equal and integral acess. A country of continental dimensions, with an estimated population of 207.7 million inhabitants in 2017 and demographic pyramid tending to a populational senility, Brazil proposes to maintain healthcare services in increasing prices. Nevertheless, in practice, it can not hold a high level service. In the other hand, some islands of excellence persists, and are world references - amonsgst them, the STI/AIDS and Viral Hepatitis from the Ministry of Health. In this aspect, even with educatioal campaigns, and the acess to prevention methods, it can be verified the incidence increase of cases in the younger population. **Methods:** The HIV infection diagnosis, in Brazil, is governed by an especific guideline that determins the use of, at least, one of the Six Flow Chart. In our Lab, it has been used the Sixth Flow Chart. It consists in a fourth generation screening test (Abbott microparticles immunoassay by chemiminescence for the qualitative and simultaneous detection of anti-HIV 1 antibodies - M and O Groups- and/or Type Two, and the HIV p24 antibody in human serum and plasma), also, the Western blot confirmatory test (New Lab Blot BIORAD). The last, detects the multiple antibodies against each one os th viral proteins. In the study, it has been used our database from 2017, in which it was made 58,921 HIV screening tests. The reactive results incidence of 0.17% in total was stratified by age range and gender. **Results:** In the populational stratification, it was possible to observe some outstanding characteristics. 74.87% of the patients submitted to HIV test were female, and 96.80% of them were between 18 and 69 years old. The reactive results incidence, without segmentation, was 0.17%. Young men between 18 and 39 years old presented a amount of reactive result three times superior than the total population (0.57%). Lastly, men between 50 and 85 years old demonstrated an incidence two times superior than the population (0.41%). **Conclusion:** Female patients are the majority that submit to HIV test. One of the reasons is the fact that it is required in Prenatal Exams. In addition, another reason is that usually men neglect healthcare services, becoming a populational blind spot. Moreover, without generalizing, UNAIDS studies point to an active sexual life more and more precocious, without the use of preservative and the multiple partners. This facts illustrate the faces of men behavior, and corroborate to an elevated reactive HIV results incidence in young people. Also, the advent of erectile dysfunction medicines increases the incidence in over 50-years-old men. Thus, women are mostly affected as a consequence of these practices. **References:**1. Secretaria de Vigilância em Saúde. *Manual técnico para diagnóstico da infecção pelo HIV.* Ministry of Health, Third Edition, Brasília-DF, 2016.2. *HIV Prevention in the Spotlight. An analysis from the perspective of the Health Sector in Latim America and Caribbean*. UNAIDS, 2017.

B-052

Incidence Of Sorological Diagnosis Of Zika Viruses In Young Women During The Year 2017 In Private Laboratory And The Relationship With Sequels In Newborns

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Background: The Zika virus is an arbovirus, from the genus Flavivirus, belonging to the same group as Yellow Fever, Dengue, Japanese Encephalitis virus, among others. Discovered in 1947, it was isolated from monkeys samples, in Uganda (East Africa), through a monitoring network of wild yellow fever in the Zika Forest. In April 2015, the first case of autochthonous transmission was confirmed in Brazil. It was a new aggravation for pregnant women, and defined by scientific consensus, as one of the viral causes of congenital microcephaly, with possible links with other neurological disorders, besides cases reported by association with Guillain Barré Syndrome. Data from the Ministry of Health of Brazil indicate 3,037 cases of growth and developmental changes from November 2015 to December 2017, associated with infection by Zika Virus. **Methods:** In our service, we use the ELISA test (EUROIMMUN) for the indirect detection of the virus. It is based in the research of IgM antibodies. The techiques of Molecular Biology are also widely used, where the PCR (Polymerase Chain Reaction) identifies the viral DNA in samples. The use of rapid tests, according to the doctor discretion, is also a rapid and highly sensitive diagnostic tool, through immunochromatography by qualitatively detecting specific immunoglobulins. In this study, we used our database from the year of 2017, in which we performed 1,077 IgM serologies for Zika Virus, with an incidence of reagent results for Zika of 1.02% in total and stratified by age and sex. **Results:** In the populational stratification, it was possible to observe some strinking characteristics, 64.62% of the pacients submited to the serology for the Zika Virus were female, 96.98% of them were between 18 and 49 years old, the age group with the highest prevalence of pregnancies. In women from 18 to 49 years old, the incidence of positive results was 1.19%, slightly above the total incidence. In men, there were only 3 cases of reactive results in 381 tests performed. **Conclusion:** Zika Virus infection in pregnant women is a serious public health problem in Brazil due to the correlation between infection and important sequelae in newborns. Brazil is a country with an expressive territorial area, elevated population density and a tropical climate with high precipitation rates. Therefore, this facilitates the proliferation of the vector Aedes Aegypti, requiring indistinct involvement among all to face this adversity. Consequently, the Ministry of Health should promote public policies to prevent new cases, besides epidemiological data and generation of scientific knowledge. **References:**

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B-053

Changing from a modified to an unmodified testing of Hepatitis C Viral Loads: An evaluation of the Roche Cobas 4800 system Cobas HCV kit.

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Introduction

Hepatitis C virus (HCV) is one of the several viruses known to cause viral hepatitis. Nucleic acid testing (NAT) is a widely accepted confirmatory method to determine HCV infection. Assessment of HCV viral load provides better clinical utility in measuring baseline viraemia and improving the efficacy of antiretroviral treatment. As part of a response-guided therapy, a periodic assessment of HCV at specific intervals would allow further personalize treatment plan to become possible. A comparison study was done between the Cobas HCV (Roche Diagnostics, Switzerland) assay using the Cobas 4800 system, a complete and automated assay, against our current method, the Cobas TaqMan48 HCV v2 with sample preparation performed on the Qiagen EZ1 (Qiagen, Netherlands).

Material and methods

A total of 56 cell-free anonymized serum and plasma samples, with HCV viral loads ranging from undetected to Log^{10} 7.22 IU/mL, were tested on the Cobas 4800 system. Precision studies were derived from quality control materials at HCV viral loads of Log^{10} 2.38 and Log^{10} 6.36IU/mL. The data was assessed quantitatively using regression analysis and Bland-Altman bias plots. Qualitative assessment was made using a binary matrix to derive specificity and sensitivity of the assay. The limitation of the detection was derived from serial dilution of known high titer samples with negative serum.

Result

The Cobas 4800 HCV assay produced diagnostic sensitivity and specificity of 100% compared to the TaqMan48 method. Regression analysis showed a correlation of 0.8722x + 0.8805 (R=0.9697). A (-) Log^{10} 0.33 difference between the two method means. Precision studies based on the manufacturer's quality control material gave a standard deviation 0.06 (HCV; Log^{10} 2.38IU/mL) and 0.05 (HCV; Log^{10} 6.36 IU/mL). Limit of detection was determined to be 16.1 IU/mL, which was slightly higher than the manufacturer's declaration of 15.0 IU/mL.

Conclusion

The performance of the Cobas HCV assay on the Roche Cobas 4800 system was comparable to the current Cobas TaqMan HCV v2 method. Accuracy of viral load determination, instrument precision and performance limits were indistinguishable between both methods. However, the current semi-automated modified method of sample preparation on the Qiagen EZ1 and manual pre-PCR preparation for the TaqMan48 is prone to contamination and mistakes can occur. Almost throughout the whole process, operator involvement is required and subject to inter-operator variation. By adopting the automated and unmodified Cobas 4800 assay, operator intervention is only required at the start of the process and to transfer the instrument-prepared PCR plate between instruments. This allows a significant amount of walk-away time while maintaining high standards of quality and better utilization of operator skills for more complex operations.

B-054

Temporal patterns of troponin I and Jarisch-Herxheimer reaction in *Cryptococcus gattii* infection

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Background: Over the past two decades the fungal pathogen *Cryptococcus gattii* has emerged as a cause of disease in humans and animals in the Pacific Northwest. We report on a case of *C. gattii* infection in Skagit County, WA complicated by Jarisch-Herxheimer (JHR) reaction and increased cardiac troponin. **Case Report:** A 46-year-old woman was seen at a local hospital with febrile illness. Past medical history was unremarkable. She had an increased heart rate and blood pressure. A urinalysis was within normal limits. Chemistry showed elevated renal (BUN = 35 mg/dL; creatinine = 1.2 mg/dL) and liver (AST = 125 U/L; ALT = 180 U/L) enzymes, high glucose (154 mg/dL) and normal electrolytes. The patient was non-reactive for acute hepatitis panel and HIV. A base troponin I was normal (0.04 ng/mL). Hematology showed leukocytosis (25,000/mm3) and thrombocytopenia (98/mm3). A cryptococcal antigen assay (Cr-A) on serum was positive. A semi-quantitative analysis returned a 1:80 titer. A working diagnosis of cryptococcosis was made. A computed tomography (CT) scan of her chest showed a 50-mm lesion in the upper-right field of her lung. Lung biopsy pathology showed scattered fungal spores and positive periodic acid-Schiff (PAS) staining. A lumbar puncture to rule out asymptomatic CNS involvement returned a negative Cr-A on CSF. Fluconazole therapy was started but a clinical worsening of symptoms developed in 6 hours complicated by J-HR - temperature, blood pressure and platelet count decreased rapidly. At 12 hours chest and low back pain developed, and a troponin was 0.16 ng/mL. At 14 hours a second troponin had increased to 0.24 ng/mL. The patient was transferred to the ICU unit. Over next 96 hours the thrombocytopenia improved (112/mm3), troponin levels normalized (less than 0.07 ng/mL), and febrile illness and angina resolved. Fourteen days after onset of the illness, the patient's hematology and troponin were within normal reference range, the triage of transient events related to the JHR were absent. The Cr-A was still positive and returned a titer of 1:320. For the next 16 months the liver enzymes (AST,ALT) remained slightly elevated throughout the course of antifungal treatment. At 18 months post treatment the Cr-A was negative and the patient's liver enzymes normalized soon after therapy was halted. **Conclusion:** The JHR is a well-known complication of antimicrobial but not antifungal therapies. Studies have shown that cytokines, namely tumor necrosis factor and interleukins appear in the circulation transiently and correlate with symptom severity in pathogenesis of cryptococcosis. Antibodies against inflammatory cytokines have been shown to decrease the JHR. Sepsis that results from the presence of infectious organisms is frequently associated with changes in these inflammatory mediators. Elevations in cardiac troponin in patients with sepsis is common. The potential causes of troponin release during sepsis include decreased cardiac integrity, fungal polysaccharide capsule destruction and thrombotic dysfunction. This is the first reported occurrence of elevated troponin and a JHR reaction associated with antifungal treatment for cryptococcal disease.

B-055

Development of non-amplification DNA detection method for MPB64 in *Mycobacterium tuberculosis* complex

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Background: Even though the definite diagnoses for many diseases have been long believed to be performed with PCR, there are many issues to be solved in PCR. For example, they are (1) non-specific or false positive amplifications, (2) volume limit for a target sample, (3) deactivation of enzymes used, (4) complicated techniques, and so forth. The non-specific or false positive amplifications occur due to an excess DNA input, long targets or contamination. A sample amount used is about 1 μL, showing that at least 1000 copies have to be included in a 1 mL volume. This low concentration brings the false negative data. The deactivation of enzymes leads the deterioration of amplification efficiency. Further, the PCR techniques are complicated even at present. Thus the placement method of PCR is strongly needed without DNA amplification. In the present study, we propose a new method for detection of nucleic acids without any amplification and detect the gene of MPB 64, a specific protein in *Mycobacterium tuberculosis* (TB) complex. Methods: We have developed a new method using a combination of hybridization and thio-NAD cycling. The cDNA probes linked with FITC hybridized to the target sequences in the MPB64 gene. The anti-FITC antibody linked with ALP was applied, and then a cycling reaction was conducted by a dehydrogenase (3α-hydroxysteroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androsterone phosphate). That is, our new method is referred to as "non-amplification nucleic acid detection method". The single strand, the double strand and the DNA plasmid were used for the MPB64 gene detection, whereas *Mycobacterium avium* and *Mycobacteri*um *intracellulare* were used for a non-TB control sample. Two or four probes were prepared for each strand. Results: We obtained that the limit of detection was 3.7×10^5 copies/assay (i.e., 4.2×10^3 copies/μL), and that the limit of determination was 1.3×10^6 copies/assay (i.e., 1.4×10^4 copies/μL) for the single strand of MPB64. Using the double strand, the limit of detection was 1.3×10^6 copies/assay (i.e., 1.4×10^4 copies/μL), and the limit of determination was 4.2×10^6 copies/assay (i.e., 4.7×10^4 copies/μL). Using the plasmid, the limit of detection was 7.0×10^5 copies/assay (i.e., 7.8×10^3 copies/μL), and the limit of determination was 2.3×10^6 copies/assay (i.e., 2.6×10^4 copies/μL). We did not observe any response to the non-TB control samples. Conclusion: Because the protocol of washout is included in our method and the measurement volume can be larger than PCR, the possibility of false positive or negative results is decreased. The deactivation of enzymes can be avoided within the condition as described above. Therefore, our new method overcomes every difficulty of PCR. Furthermore, we should add one comment on the comparison with the Interferon-Gamma Release Assays (IGRA). The cost of our detection method is much less than IGRA.

B-056

Analytical validation of real-time PCR assay for detection of West Nile virus: the benign of laboratory diagnosis

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Background: Since its discovery in 1937, the West Nile Virus (WNV) has expanded beyond its known geographical reach and caused disease in humans on several continents. It is currently the most common cause of neurological diseases caused by arboviruses worldwide. WNV is an arbovirus of the *Flaviviridae* family, which has its RNA genome of approximately 11,000 kb. Maintained in nature in an enzootic transmission cycle between birds and mosquitoes, WNV can also infect humans and other vertebrates. Such infections are usually mild or subclinical, with 80% of human infections being asymptomatic. In the diagnostic laboratory WNV can be inferred by ELISA, however, this assay is limited due to the difficulty in the differentiation between WNV and others virus. Molecular methodologies such as real-time PCR have been indicated since they are highly sensitive and specific for the detection of RNA viruses, including WNV. **Objective**: To describe the analytical validation of a real-time PCR assay for detection of West Nile Virus using commercial control. **Methods**: The primers and probes were designed from two conserved regions of the WNV genome (New York 1999 WNV isolate): 3' noncoding region (NCR) and envelope region (ENV). Performance of assay was evaluated using commercial quantified positive controls and the parameters of analyze included: (i) Determination of threshold (Dilutions of RNA which were run in triplicate); (ii) Analytical sensitivity (Limit of detection in replicates with concentration of RNA in the range of 1.250 to 19 copies/µL); (iii) Intrassay and interassay precision (Test of RNA in triplicates with one concentration at the limit of detection, one with a concentration 20% above the limit of detection, and one with a concentration 20% below on the same day and 3 different days); (iv) Analytical specificity (Interference study with Dengue virus, Chikungunya virus, Zika virus, Yellow fever virus); (v) Test of spike in (A negative sample was spiked with positive control). **Results**: The determination of the detection threshold remained within the range of linearity of a standard curve with a coefficient of variation (CV) of 0.99. The detection limits were 39 copies/µL for both the targets (95% confidence interval). The regression equations obtained show good amplification conditions with positive correlation between the variables, with a coefficient of determination (r^2) of 0.99 and amplification efficiency of 94% (NCR) and 103% (ENV). The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility. No cross-reactivity was observed. The spiked sample presented positive results with a minimum value of 3.125 copies/mL of sample. **Conclusion**: This analytical validation provides data indicating that the specificity and sensitivity of the assay for a WNV detection system fulfilled the criteria requested by international guidelines. This study was not tested in real clinical samples therefore, before implementation of the assay in routine diagnostic laboratory; a clinical study is needed to establish the method in clinical and operational settings. Therefore, further studies will be performed to more effectively evaluate the possibility of using this method in routinely detection of WNV in various clinical samples.

B-057

Yellow Fever: what has been happening in Brazil?

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Background: Yellow Fever (YF) is a zoonotic flaviviral disease caused by the Yellow Fever virus (YFV), which is carried by the vector mosquitoes *Haemagogus* and *Sabethes* (sylvatic cycle) and *Aedes aegypti* (urban cycle). This disease is a reemerging, zoonotic, noncontagious viral hemorrhagic disease endemic and epidemic in tropical regions of South America and Africa to Africa and South America; outbreaks occasionally occur among human and nonhuman primates. According to the World Health Organization (WHO), YF remains an important public health problem and has been estimated at over 200,000 cases per year worldwide, causing 30,000 deaths. The true incidence of YF infection is unknown due to insufficient reporting and ground surveillance. Therefore, there is an urgent need to detect and study the prevalence of YFV in Brazil and regions in order to contribute to the implementation of public health policies in Brazil. **Objective**: To describe the behavior of Yelow Fever virus infection in Brazil and federative units during period of August 2017 to January 2018. **Methods**: This was a retrospective study, carried out through consultation of laboratory test results stored in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) web LIS. All results of a Yellow Fever IgG and IgM obtained and released from August 2017 to January 2018 were compiled. Epidemiological data such as gender, age and region of the country of Yellow Fever IgG and IgM patients were statistically analyzed. **Results:** A total of 516 patients from all over the country were evaluated between 2017 and 2018. There was a predominance of patients from the Southeast region (75.4%), home of the laboratory, followed by the South (8.3%), Northeast (7.0%), Midwest (6.2%), and North (3.1%). The rates of positivity for Yellow Fever IgG and IgM were 25.8% and 2.7%, respectively. Among IgM positive cases, 7.1% were children (under 20 years), 92.9% adults (between 20 and 60 years). There was a male predominance in adults and female predominance in children patients. 92.9% of these cases were from Southeast region, and 7.1% were from Midwest. In Southeast, serology was mostly positive in men while in the Midwest, the positivity was higher among women. Considering just January 2018 the number of positive IgM cases was 3.67% more than all cases of the period analyzed of 2017. **Conclusions**: The high levels of positive IgG antibody may be attributed to vaccine impact, therefore the data of IgG antibody was not detailed in this study. The epidemiological profile of seropositive for YF IgG and IgM antibodies assisted by IHP was similar to the Ministry of Health regarding the Brazilian population. A complex combination of ecological, social, and behavioural factors may help to explain the severity and efficient spread of the YFV in Southeast Brazil, particularly its dissemination to the Atlantic coast. Despite being an area where routine immunisation is recommended and there is good vaccination coverage in the young population, the epidemic reached and hit with severity the rural area, due to poor vaccine coverage among adults.

B-058

Comparison of two processing methods to traditional nucleic acid extraction for qPCR in faecal samples

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Background: Gastrointestinal infections cause a huge impact on world health. Real-Time PCR (qPCR) has become a routine and robust technique for improving the diagnosis. Commercial extraction procedures for obtain nucleic acid (NA) necessary to carry out the amplification are usually expensive, time consuming and utilize dangerous reagents for human health. This study evaluated two quick extraction processes in faecal specimens to improve a rapid diagnosis in combination with qPCR. **Methods**: This study was carried out in 78 faecal samples from patients with clinical suspicion of gastrointestinal disease (collected in July 2017). NA was isolated with three different types of extraction procedures. The reference standard (RS) was a commercially available kit using a silica-based matrix, VIASURE RNA-DNA Extraction Kit (Certest Biotec, Spain). Two quick processes were compared to RS, VIASURE Lysis Buffer (LB) which has been recently developed by Certest, and transport medium compatible with PCR reagents MSwab™ (MS) from Copan (Italy). Both obtained NA using a simple and rapid boiling procedure. qPCR assays run on thermocycler Cobas Z480 (Roche Diagnosis) using VIASURE gastrointestinal panel (Certest). **Results**: A total of 111 pathogens (53 bacteria, 18 parasites and 40 viruses) in 73 positive samples were diagnosed. Co-infections were identified in 40 % total specimens. The difference Cq values (ΔCq) between RS and LB in bacteria and parasite were in average less than 3. For viruses, the difference was between 3-6. 8 positive samples were no detected with LB procedure, 2 of them were considered random positives. In regard to MS, ΔCq compared with RS in bacteria and parasite was in average ≥3. For viruses, in almost all cases were considerably greater than 3. In Astrovirus and Norovirus GII the difference was >10 and Norovirus GI positive samples were not detected. 23 positive samples were no identified with MS procedure, 8 random positives. **Conclusions**:

1- LB is a simple and rapid procedure, valid for universal NA isolation in fecal specimen and compatible with qPCR, which improve a rapid diagnosis.
2- In comparison with another quick treatment, LB identified most microorganism and the Cq values are closest to RS.

B-059

Comparison of HDV RNA level and severity of liver disease among subjects with HDV/HBV infection.

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Background: Hepatitis B virus(HBV) and hepatitis delta virus(HDV) superinfection often show severe chronic hepatitis, but sometimes it lacks any symptoms of liver disease in some patients. Mongolia is one of the countries with the highest HDV prevalence in the world. According to study conducted in 2015, 10.6% of apparently healthy Mongolian population tested positive for HBsAg and 61% of HBsAg positive subjects were positive for HDV-RNA. Another study by Japanese group found correlation between circulating HDV RNA level and liver injury. We assessed 46 subjects whose serum HBV DNA and HDV RNA levels were quantitated by RT-PCR to see if there is any correlation of HDV RNA level with the subjects' alanine aminotransferase (ALT) levels and their liver damage status (ASC-asymptomatic, CH-chronic hepatitis, LC-liver cirrhosis) diagnosed by doctors. **Methods:** We conducted a retrospective study to review records for 46 subjects (age 26-72, female 22, male 24) who were tested positive for both HDV RNA (Genesig, UK) and HBV DNA (Abbott, USA) at Gyals Medical Center in Mongolia, between 2016-2017. All data were analyzed by STATA statistical analysis software. **Results:**
There was no statistically significant correlation between the severity of liver damage and level of HDV RNA (**Figure 3)**. However, subjects with LC diagnosis showed weak statistical correlation (p=0.088) between ALT level and HDV RNA level (**Figure 2)**. When we compared HDV RNA levels with HBV DNA levels in ASC, CH and LC subjects, ASC subjects had low level of HBV replication (under LOD) and higher level of HDV RNA replication (**Figure 1)**. The levels of HBV DNA in serum did not differ among the 3 groups. **Conclusion:** In this study, weak correlation was observed between serum HDV RNA level and ALT level among LC subjects and it could mean that HDV RNA may play role in liver pathogenesis, as confirmed by previous studies. An interesting trend was seen among ASC subjects: low level of HBV replication was observed with higher level of HDV replication, which calls for broader study involving bigger subjects pool. **Figure 1.** Relationship between levels of HDV RNA (copies/ml) and of HBV DNA (IU/ml). ASC-asymptomatic carriers, CH-chronic hepatitis, LC-liver cirrhosis.**Figure 2.** Relationship between level of HDV RNA and level of alanine aminotransferase (ALT), in serum of liver cirrhosis (LC) subjects.**Figure 3.** Levels of HDV RNA in severity of liver disease. ASC-asymptomatic carriers, CH-chronic hepatitis, LC-liver cirrhosis.

B-060

Cytomegalovirus Quantitative Detection: a Novel, Rapid and Sensitive Ready-To-Use Real-Time PCR-Based Kit

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Background: Human Cytomegalovirus (CMV) is a ubiquitous human-specific DNA virus, belonging to the *Herpesviridae* family. CMV infection is usually asymptomatic and is common even in the general immune-competent population, with an infection rate of 50-80%. In immunocompromised patients that undergo transplantation the CMV infection rate is even higher, being an important cause of morbidity and mortality. Congenital CMV infection is the most common congenital infection worldwide and is the leading non-genetic cause of sensorineural hearing loss in children. Respect to traditional techniques of virus isolation, molecular methods demonstrated to be a rapid and sensitive alternative for virus detection. The aim of this work was to evaluate the performance of a new quantitative freeze-dried and ready-to-use assay designed to detect CMV DNA in human samples. **Methods:** A novel quantitative Real-Time PCR based-assay was developed as a ready-to-use test with specific sets of primers and probes able to amplify two different conserved regions within CMV genome. A third set of primers and probe, specific for a Human Beta Globin gene fragment, was used as an internal control. These three sets were combined in a lyophilized ready-to-use mix and all the targets were co-amplified and detected using different Real-Time PCR instruments. In the present study, several samples obtained from San Raffaele Hospital in Milan and previously diagnosed as positives and negatives with the "CMV ELITe MGB® Kit" (ELITech Group) were investigated. Real-Time PCR reactions were performed using DNA extracted from plasma, swab, bronchoalveolar lavage or biopsy. **Results:** This new quantitative freeze-dried ready-to-use assay showed to be specific for CMV, giving robust and accurate amplification of CMV target regions with a sensitivity and a specificity of 100%. All the tests performed with this assay confirmed the results obtained at San Raffaele Hospital and indicated a Limit of Detection below 10 genome copies per reaction, thus reaching the same LoD of CMV ELITe MGB® Kit. **Conclusion:** This novel Real-Time PCR assay proved its effectiveness for the quantitative detection of CMV DNA in clinical samples. Its high-sensitivity and specificity, associated with the ready-to-use feature and room temperature storage, would easily improve the early and correct management of CMV-affected patients.

B-061

A compact PCR system for rapid and sensitive detection of Middle East respiratory syndrome-coronavirus

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Middle East respiratory syndrome (MERS) is a zoonotic viral respiratory disease with dromedary camels as the major reservoirs. Rapid identification of the etiological agent, MERS-coronavirus (MERS-CoV), near patients could greatly facilitate efficient disease management and control. The POCKIT™ COMBO system (GeneReach), including a compact automatic nucleic acid extraction device (taco™ mini) and a simple insulated isothermal PCR device (POCKIT™), enables pathogen detection at settings close to points of need. Clinical performance of a qualitative matrix MERS-CoV RT-PCR method targeting both *upE* and *ORF1a* marker genes (LoD95%, 30 and 17 genome equivalents, respectively) on the POCKIT™ system were evaluated. Clinical performance of the index matrix RT-PCR was compared to a commercial real-time matrix RT-PCR (RT-qPCR) targeting the same genes (RealStar® MERS-CoV RT-PCR Kit; Altona Diagnostics) on a RotorGene system (Qiagen) by testing 102 nasal swab samples. Positive results were derived from positive detection by one of the two markers. Nucleic acids extracted by MagNA Pure system (Roche) or taco™ (GeneReach) were tested by the two RT-PCR methods in parallel. 2x2 contingency analysis of the results shows that 40 were positive and 58 negative in both methods, while one was reference RT-qPCR negative/index RT-PCR positive and three were reference RT-qPCR positive/RT-PCR nagative. Interrater agreement calculated by kappa test was 96.08% (CI95%, 91.62 - 100%; $\kappa = 0.92$), indicating that the index matrix RT-PCR and the reference RT-qPCR had excellent agreement. Components of the POCKIT™ Combo system have also been integrated into an automatated sample-to-results device to help reduce hands-on time and enhance test consistency. The reagent is ready in a lyophilized format. Providing detection performance comparable to laboratory real-time RT-PCR system, the MERS-CoV RT-PCR on the POCKIT™ systems have potential to serve as an effective point-of-need tool for rapid detection of MERS-CoV.

Table 1. 2x2 contingency analysis: comparison between reference real-time RT-PCR system and index RT-PCR on the POCKIT™ system

		MERS-CoV real-time RT-PCR		
		Positive	Negative	Total
MERS-CoV RT-PCR (POCKIT™ system)	Positive	40	1	41
	Negative	3	58	61
	Total	43	59	102
Agreement, 96.08%; CI95%, 91.62 ~ 100%				

B-062

New diagnostic markers for differentiation of acute and convalescent human cytomegalovirus infections

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Background
Human cytomegalovirus (HCMV) is the most common viral pathogen of congenital infections. The risk of virus transmission from mother to foetus is highest in acute primary infection during pregnancy. In affected children, HCMV can cause severe complications. Serological diagnosis of early primary infections, however, is challenging for two reasons: 1) presence of anti-HCMV IgG is indicative for primary infection only if seronegativity has been documented in a previous sample; 2) an-

ti-HCMV IgM antibodies may persist and can be associated with different clinical settings, such as acute primary, convalescent primary or recurrent infection. Here, we present two novel markers, anti-HCMV p52 IgM and anti-HCMV gB IgG, for better differentiation of acute and convalescent phases of HCMV infection.
Material & Methods
A commercial panel for anti-HCMV seroconversion (Biomex, Germany) comprising 25 follow-up samples covering 124 days from a male was analysed. In addition two patients from a reference laboratory (Lübeck) were tested: Patient 1 presented with fever and fatigue; serum samples were taken one, two, four, five and seven months after onset of symptoms. Patient 2 is a female whose serum samples were taken 20 months before and during pregnancy (week 8 and 33). Samples were tested for anti-HCMV IgM and IgG as well as anti-HCMV p52 IgM and anti-HCMV gB IgG using ELISA (Euroimmun AG, Germany). For detection of anti-HCMV p52 IgM and anti-HCMV gB IgG, new recombinant antigenic substrates are applied. ELISA were conducted according to manufacturer's instruction.
Results
The commercial panel demonstrated seroconversion of anti-HCMV IgM and IgG around day 35 of monitoring as well as positivity for anti-HCMV p52 IgM between day 35 and day 85. When anti-HCMV p52 IgM declined below cut-off, anti-HCMV gB IgG appeared. Patient 1 revealed high titer anti-HCMV IgG in all samples. Anti-HCMV IgM and anti-HCMV p52 IgM were initially present but turned negative three to four months after onset of symptoms. At that time, seroconversion of anti-HCMV gB IgG was observed. Patient 2 was tested negative in all assays before pregnancy, but showed high titer anti-HCMV IgG and anti-HCMV gB IgG, when she was eight weeks pregnant. Anti-HCMV IgM and anti-HCMV p52 IgM were negative. Equal results were obtained in pregnancy week 33.
Conclusion/Discussion
The antibody courses in the commercial panel and patient 1 support the concept of anti-HCMV p52 IgM being a putative marker for acute HCMV infection, while detection of anti-HCMV gB IgG is indicative of a convalescent phase. IgG seroconversion of patient 2 implies that the woman got infected with HCMV. However, according to the concept, infection is likely to be convalescent at the time of second blood withdrawal (anti-HCMV p52 IgM negative, anti-HCMV gB IgG positive) and thus, the risk of virus transmission to the eight weeks old foetus would be low.

B-063

The time course of calprotectin release from human neutrophil granulocytes after challenge with E. coli

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Background: Plasma calprotectin has previously been reported as a promising biomarker for sepsis. Calprotectin is released from neutrophil granulocytes upon inflammatory activation. However, little is known about how promptly calprotectin is released from these cells in response to encounters with bacteria or pathogen associated molecular patterns (PAMP). A new turbidimetric method (PETIA) that offers a relatively inexpensive analysis of calprotectin with rapid turn-around times from sampling to laboratory results was launched last year. Rapid turn-around-time for analysis and early release of calprotectin upon inflammation and/or infection suggest that calprotectin can become a useful biomarker with widespread clinical use. The aim of the present study was to elucidate the kinetics of calprotectin release from blood neutrophils, exposed to E. coli. We also analyzed other inflammatory mediators with known kinetics as comparison, and kidney injury molecule 1 (KIM-1) as a non-blood cell inflammatory biomarker. **Methods:** Whole blood samples were exposed to E. coli bacteria in vitro. Blood samples were collected after 0, 1, 2, 3 and 4 hours. Plasma calprotectin was analyzed with a particle enhanced turbidimetric immunoassay (PETIA) while tumor necrosis alpha (TNF-α), interleukin-6 (IL-6), neutrophil gelatinase-associated lipocalin (NGAL) and KIM-1 were analyzed by ELISA. **Results:** When the blood cells were exposed to E. coli, the calprotectin levels began to increase within 1-2 hours after the exposure. IL-6, TNF-α and NGAL increased above baseline at 1 hour after bacterial exposure while levels of KIM-1 were beyond detection limit in almost all plasma samples. **Conclusion:** Our data demonstrated that calprotectin increases early in response to bacterial challenge. Given the logistic advantages of the calprotectin PETIA analysis, this biomarker may be of interest for early diagnosis of bacterial infections.

B-064

A brazilian case report of yellow fever infection

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Background: Yellow fever (YF) is a mosquito-borne viral hemorrhagic fever, which is a serious and potentially fatal disease with no specific antiviral treatment that can be prevented by an attenuated vaccine. Since December 2016, Brazil is affected by an unusually large and expanding yellow fever outbreak, with over 3,500 suspected cases reported and several hundred deaths. In early 2017, the Brazilian Ministry of Health reported outbreaks of this disease in several eastern states, including areas where yellow fever was not traditionally considered to be a risk. In January and February 2018, 88 cases of YF were recorded in the state of Minas Gerais, Brazil. Descriptive epidemiological evidence suggests that the outbreak so far shows a sylvatic transmission pattern with human infections being acquired from non-human primates (NHP) via forest-associated mosquito species. However, recent research has identified urban mosquito vectors to be competent for transmission of yellow fever virus (YFV), suggesting a risk of re-emergence of urban YF in Brazil. **Objective**: To report the laboratory profile of a brazilian patient with yellow fever infection which was confirmed by molecular method (Real Time PCR). **Methods**: The laboratory tests requested were carried out in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil according to care routine. **Results**: A 49-year-old man was admitted to a hospital in January 11, 2018 because of high fever with severe headaches, fatigue and weakness. Alpha 1 antitrypsin was within the normal range, Ceruloplasmin was significantly decreased, which may be indicative of hepatic degradation. Antibody Anti LKM-1, autoantibodies against Smooth Muscles (ASMA) and Cell Nuclei (ANA) were normal and Autoimmune liver disease and rheumatic diseases were excluded, respectively. Besides that autoantibodies against Granulocyte Cytoplasm (cANCA/pANCA) were normal and Wegener's granulomatosis, glomerulonephritis, primary sclerosing cholangitis, ulcerative colitis, Crohn's disease were also excluded. In the patient´s infectious examination, serologic laboratory tests (IgM antibody) of Anti Chikungunya, Anti Cytomegalovirus, Anti Dengue, Anti Epstein Barr, Anti Hepatitis A, B, C and E, Anti-Herpes Virus, Anti HIV, Anti Leishmaniasis, Anti Leptospirosis, Anti Parvovirus B19, Anti Zika Virus were negative. The clinical course presented fatal complications and the patient died 7 days of the onset of symptoms. C**onclusion**: The case reported refers to a patient with history of stay in the municipality of Brumadinho, state of Minas Gerais, Brazil, an area where the circulation of yellow fever virus is currently occurring. Moreover, the case has no history of yellow fever vaccination. Although Brazilian health authorities have swiftly implemented a series of public health measures in response to the outbreak, including mass vaccination campaigns, it may take some time to reach optimal coverage in these areas given the large number of susceptible individuals.

B-065

Development of a Comprehensive set of Zika Virus Reference Materials for Validation and Evaluation of Performance of Serological and Molecular Assays.

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Background: Outbreaks of Aedes mosquito borne Zika have occurred in areas of Africa, Southeast Asia, Pacific Islands, Brazil and continues to spread rapidly through many countries of the Americas. Zika virus causes the infectious Zika fever and has been linked to Guillain-Barre syndrome and neurological birth defects. In response to the outbreak, several serological and PCR-based Zika assays have been developed and approved under FDA's Emergency Use Authorization. Clinical laboratories and diagnostic test developers need robust positive reference materials and panels that can evaluate these assays across the entire reportable range for sensitivity and linearity. SeraCare has developed a suite of products that not only fulfills the need for specificity in current Zika assays that is critical in isolating the proper virus to determine treatment; but also provides safe to handle, virus-like material that can be used as positive control in Zika diagnostic assays. **Methods:** SeraCare has developed three new products; AccuPlex™ Zika RNA positive reference material, AccuSpan™ Zika RNA Linearity Panel and the AccuSet™ Zika IgM Performance Panel. AccuPlex Zika RNA Reference Material and AccuSpan Zika RNA Linearity Panel is formulated using SeraCare's recombinant virus technology and intended for use with nucleic acid test methods for external quality control that detect the ZIKV 2007 strain. The entire genomic RNA sequence is packaged into specially modified recombinant Alpha viral vectors and diluted in defibrinated human plasma (DHP). The AccuSpan

Zika RNA Linearity Panel consists of six positive members, each prepared from the preceding member by diluting recombinant Zika Virus with Zika RNA negative diluent in serial ten-fold dilutions beginning at member 1, the highest positive (target concentration of 1.0E+06 copies/mL verified by dPCR analysis), and ending with member 6, the lowest positive. The diluent was prepared from normal human defibrinated plasma negative for Zika RNA. AccuSet Zika IgM Performance Panel is a 10-member performance panel consisting of undiluted, naturally occurring undiluted plasma samples with reactivity ranging from negative to positive for Zika IgM and IgG antibodies. **Results:** AccuPlex Zika Reference Material is a ready-to-use product that is formulated for use with PCR- based assays that detect the ZIKV 2007 strain. The AccuSpan Zika RNA Linearity Panel can be used to evaluate the dynamic range of Zika RNA assays, identifying consistency over a linear range, verifying lot changes and performing linearity studies. AccuSet Zika IgM Performance Panel is a comprehensive panel consisting of real patient samples for serological test methods for Zika IgM and Zika IgG. The panel members have been highly characterized and comprehensively tested on several methodologies including the CDC algorithm and therefore designed to help researchers evaluate Zika infectivity from that of related flaviviruses. **Conclusions:** SeraCare has developed a suite of Zika products for PCR based and serological test methods that enables researchers and diagnostic manufacturers to expedite the development, evaluation and validation of Zika assays.

B-066

Phenotypic Susceptibility Profile of Methicillin Resistant *Staphylococcus aureus* in the Dominican Republic

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Background. Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major challenge to clinicians. The prevalence of MRSA has increased over the past decades. The burden can vary by geography and healthcare systems. There is a paucity of data on MRSA susceptibility patterns in developing nations. Local susceptibilities have an important role for the selection of empiric treatment choices in patients with suspected MRSA infections and in those with beta-lactam allergies. We seek to define the prevalence and resistance profile of SA in the Dominican Republic (DR). **Methods.** This is a retrospective review of resistance patterns of *S aureus* (SA) isolates from a clinical laboratory in the DR (Amadita Laboratories). Amadita provides services nationwide. Data collected from 2016-17 included organism sensitivity patterns and geographic location. Automated susceptibility testing (Vitek®2, bioMérieux) was used for susceptibilities and clindamycin inducible resistance testing. **Results.** Of 1674 samples of SA, 869 (52%) were MRSA and 805 (48%) were susceptible to methicillin (MSSA). MRSA resistance to tetracycline was high (82%). Clindamycin resistance was more likely to be inducible (19% vs 1.5%). Eight isolates were resistant to vancomycin (VRSA) and 29 isolates (3.3%) had minimum inhibitory concentrations above 2. MRSA was more common in rural areas (56% vs 50%). Vancomycin resistance was more common in urban areas (2.8% vs 0.2%). Antimicrobial susceptibilities are shown in Table 1.

Table 1. Antimicrobial resistance for SA isolates by drug resistance category (%)

	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Levofloxacin	Linezolid	Oxacillin	Quinoprustin/dalfopristin	Penicillin G	TMP-SMX	Rifampin	Tetracyclines	Tigecycline	Vancomycin
All isolates	11.7	18.9	63.9	3.4	2.8	1	52	0	95	2.9	0	45.5	0	1.2
MSSA	12.4	33	38.5	2.1	1.8	1	0	0	90	6	0	58	0	1.4
MRSA	11.1	5.7	87.5	4.7	3.7	1	100	0	99.5	0	0	82.2	0	0.9

Conclusion. In this nationwide sample, MRSA was more common than MSSA. SA resistance profiles in the DR have high rates of resistance to tetracyclines. Clindamycin resistance was higher for MSSA isolates and was commonly inducible. Clinical laboratories in the region should consider routine testing of inducible resistance to clindamycin. Trimethoprim-sulfamethoxazole (TMP-SMX) and linezolid have the most optimal susceptibility profile of available oral agents against MRSA. The rise of vancomycin resistance is concerning and requires further study.

B-067

Evaluation of a Procalcitonin Assay on the Atellica IM Analyzer

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Background: Procalcitonin (PCT) is a 116 amino acid peptide that shares a common structure with the prohormone of calcitonin. Under normal metabolic conditions, calcitonin prohormone is produced by the thyroid's C-cells, where it undergoes proteolysis to yield the hormone calcitonin. Calcitonin is then involved in calcium homeostasis. Under normal conditions, plasma levels of the calcitonin prohormone have been shown to be under 0.1 ng/mL. However, during episodes of severe bacterial infection and sepsis, the level of blood-circulating PCT increases to levels generally above 2 ng/mL. In response to proinflammatory stimuli, such as bacterial infection, operation, or trauma, PCT can be produced by nearly every tissue of the body. Siemens Healthineers has developed a procalcitonin assay for the Atellica® IM Analyzer with acceptable sensitivity, precision, and linearity to aid in the risk assessment of critically ill patients for progression to severe sepsis and septic shock on their first day of intensive care unit (ICU) admission. The Atellica IM PCT Assay is an 18-minute sandwich immunoassay with a range of 0.02 to 50.00 ng/mL, and is aligned to the BRAHMS KRYPTOR assay. **Method:** The Atellica IM PCT Assay's performance was assessed with two lots of reagents. Imprecision and functional sensitivity were evaluated using two levels of control materials, a panel of five human serum precision samples, and a panel of five human serum functional sensitivity samples containing low levels of PCT analyte, tested twice a day for 20 days for a total of 80 replicates on two instruments. Linearity studies were conducted using nine human serum samples equally spaced across the assay range in a known mathematical relationship and evaluated using two reagent lots. A method comparison to the BRAHMS KRYPTOR reference method was confirmed using one lot of reagents and 265 serum patient samples with known BRAHMS KRYPTOR values. **Results:** The data obtained with the Atellica IM PCT Assay demonstrated correlation to the BRAHMS KRYPTOR method, yielding a Passing-Bablok slope of 1.02 and regression coefficient of 0.98. A 20 day precision study yielded within-lab precision CVs between 2.1% and 13.7% for the two reagent lots using samples containing between ~0.03 ng/mL and ~20.65 ng/mL of procalcitonin. Functional sensitivity for both reagent lots was ≤0.04 ng/mL. Linearity studies demonstrated that the PCT assay is linear across the assay range of 0.02 to 50.00 ng/mL. **Conclusion:** The performance of the Atellica IM -PCT Assay has been assessed and the results show an accurate, sensitive and precise method for the measurement of Procalcitonin in human serum. The Atellica IM PCT Assay is in alignment with the BRAHMS PCT Sensitive Kryptor assay and may be a valuable tool in clinical laboratories for the accurate measurement of procalcitonin in human sera. Not available for sale in the U.S. Future availability cannot be guaranteed.

Siemens Healthcare Diagnostics

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B-068

Optimization of the lamination system of urine samples in Flow Cytometry

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Background: In the iQ200 automated urine microscopy Analyzer a urine sample is sandwiched within a special fluid called "lamina", IQ lamina. This system is coupled to a digital video camera. The lamina and flowcell are key to hydrodynamically orienting the particles in the urine and increase the efficiency of cellular counters. For this lamination the reagent IQ lamina is used, with the need for a higher volume of this reagent the higher the turbidity of the sample;This study aimed to manage the urine sediment analysis routine and reduce the cost of the laminating reagent after understanding the system and measuring its performance using a graduated test tube to measure volumes used in the monthly and preventive calibration, the consumption of reagents according to the density or viscosity of the samples, either in continuous or intermittent flow, without compromising the quality of the result. **Methods:** A total of 167 turbid samples, 243 clear samples and 450 slightly turbid samples were selected during the months of March, April and May 2017. In order to eliminate the interference of amorphous particles, only non-amorphous urine samples were used. We note that the IQ 200 Sprint linearly processes up to 1000 cells / µL. We optimize a continuous flow of samples, in detriment to the intermittent flow, allowing a lower consumption of IQ lamina. **Results:** We observed a gradual reduction in the use of the IQ lamina reagent, with a reduction of 18 ml / day to 12.7 ml / day, below the target of 15 ml / day. With a saving in gallons of 0.7 / day, reaching 231.8 gallons / year. And an annual financial gain of $ 6,500

Conclusion: Managing the urine routine provided positive results, with reduced input and consequent financial gain, without compromising the quality of the exam. This was achieved by implanting a continuous stream of samples.

B-070

Ready-to-use Stabilized qPCR Assays for Detection of Zika, Dengue and Chikungunya Viruses

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Background:
Zika virus (ZIKV), Dengue virus (DENV) and Chikungunya virus (CHIKV) are arthropod-borne arboviruses transmitted by mosquitos of the *Aedes* genus. They cause similar clinical presentations, especially in the initial stages of infection, and so an early and accurate diagnosis is imperative. Polymerase chain reaction (PCR) based diagnosis has shown to be a sensitive and specific method for pathogen identification purposes. The cross-reactivity of the antibodies of these arboviruses limits the use of serology, so real time PCR is a detection method commonly used during the acute phase of the infection. Stabilization of molecular assays can overcome limitations associated with qPCR technique such us assay variability, risk of contamination and the need for cold-chain, thus enhancing the spread of qPCR technique.
Methods:
A retrospective study was performed on 66 samples from the External Quality Assesment (EQA) programs QCMD and INSTAND. Samples were collected from April 2014 to October 2017. Genomic RNA was isolated using "QIAamp Viral RNA Mini Kit" (Qiagen). Nucleic acids were analysed with two different lyophilised real-time PCR detection kits. 5 µl of sample were amplified with "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit" (Certest Biotec), while 25 µl of sample were necessary for "TaqMan Zika Virus Triplex kit (ZIKV/DENV/CHIKV)" (Thermofisher). Performance results of both kits were compared with the corresponding reports from EQA programs.
Results:
According to EQA programs reports, 12/66 samples were ZIKV positive, 24/66 were DENV positive and 19/66 were CHIKV positive. All samples were correctly detected with "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit". "TaqMan Zika Virus Triplex kit (ZIKV/DENV/CHIKV)" failed in the detection of two ZIKV and three CHIKV positive samples, while four unspecific amplifications for ZIKV and another one for CHIKV were observed.
Conclusions:
Ready-to-use lyophilized PCR detection kits represent fast, easy and useful systems for detection of tropical arboviruses, minimizing the time for reaction preparation and contamination problems, and allowing room temperature conditions for shipping and storage. "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit" offers a reliable accuracy for ZIKV, DENV and CHIKV detection from a small amount of sample.

B-071

Comparison of two rapid antigen test kits of influenza virus and rRT-PCR test results

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Background: The method of rRT-PCR was used as a standard to evaluate the sensitivity of rapid antigen detection kits of two influenza virus in screening influenza A and B viruses, providing data support for selecting suitable methods in clinical laboratories.
Methods: Totally 110 positive samples of influenza virus from fever clinic of the First Affiliated Hospital, College of Medicine, Zhejiang University were selected, including three common subtypes of sH3N2, H1N12009 and H7N9, as well as influenza B virus. Two kinds of rapid antigen detection kits of influenza virus (FDA, CE and CFDA approval) were tested. Meanwhile, the detection rate of these two kits in our hospital in 2016 was estimated.
Results: The sensitivity of reagent A to influenza A viruses sH3N2, H1N12009, H7N9 and influenza B were 65.1%, 56.5%, 29.2% and 57.1%, while sensitivity of reagent B were 65.1%, 56.5%, 29.2% and 57.1%. The sensitivity of reagent A to influenza A and B virus in the ranges with threshold cycle (Ct) values of <25, 25-30 and> 30 were 82.9% (29/35), 51.7% (15/29), 15.4% (4/26) and 87.5% (7/8), 50% (4/8), 20% (1/5). Sensitivity of reagent B in each range were 62.9% (22/35), 24.1% (7/29), 0% (0/26) and 87.5% (7/8), 12.5% (1/8), 0% (0/5). In 2016, a total of 644 positive samples of influenza A virus were detected in our laboratory, accounting for 23.9% (154/644), 35.9% (231/644) and 40.2% (259/644), respectively, in the range with Ct values of <25, 25-30 and> 30. 106 positive samples of influenza B virus were detected, accounting for 35.8% (38/106), 18.9% (20/106) and 45.3% (48/106) for each ranges. The detection rate was estimated to be 45.3% if reagent A was used to screen for samples of influenza virus tested in 2016, while be 25.1% if reagent B was used.
Conclusion: Sensitivity of rapid antigen test kit of influenza virus for different subtypes is different, higher sensitivity for higher viral loads while likely to be undetected for lower loads. Clinical laboratories should perform comprehensive performance verification before using these kits.

B-072

Contribution of Biomarkers in the Diagnosis of Sepsis in the Emergency Department

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Background: Sepsis is defined as a life-threatening organ disfuntion that is caused by a dysregulated host response to infection. Sepsis is a common condition handled in the Emergency Department (ED) and it causes millions of deaths globally each year. The research on accurate and timely diagnosis or exclude of suspected sepsis is vital to patient, which can reduce morbidity, reduces cost, and improves patient outcome. In this situation, the contribution of laboratory biomarkers is essential and so, in recent years, major efforts have been made to find biomarkers that allow early diagnosis of this disease. Procalcitonin (PCT) is the best investigated biomarker, and together with C-reactive protein (CRP), are the most frequently used biomarkers in clinical practice. Interleukin-6 (IL6) is widely investigated for its fast response to the infectious stimulus and Soluble CD14 subtype (Presepsin) is related to mediating the immune response in sepsis, but conclusive data for the application of these biomarkers are missing.The aim of this study was to investigate the diagnostic value of CRP, PCT, IL6 and Presepsin in the diagnosis of sepsis. **Methods:** 100 patients presenting at the ED with suspected sepsis were included. Blood samples were collected at first medical evaluation and CRP, PCT, IL6 and Presepsin were analyzed**.** CRP, PCT and IL6 measurements were determined in Cobas 8000 analyzer (Roche Diagnostics®) and Presepsin in Pathfast analyzer (Mitsubishi Chemical®). After diagnosis, the patients were divided is two groups: A (non-infectious etiology, localized infection or SIRS) and B (sepsis or septic shock). **Results:** The four biomarkers showed significant differences between groups (p=0.000 for PCT and IL6; p=0.034 for CRP; p=0.049 for Presepsin). The AUCs for the diagnosis of sepsis were 0.864 for PCT (p=0.000), 0.674 for CRP (p=0.044), 0.891 for IL6 (p=0.000) and 0.653 for Presepsin (p=0.047). The comparison between PCT and IL6, the two best biomarkers, did not reveal significant differences. Also no significant differences were found when comparing IL6 with the combination of CRP and PCT (AUC=0.822), the biomarkers currently used in our hospital. We developed a logistic regression model including CRP, PCT and IL6, and the AUC (0.929) was significantly higher compared to the use of biomarkers alone. The model AUC was also significantly superior to the combined use of CRP and PCT
Conclusion: Presepsin provides a limited diagnostic value for sepsis, the worst of the four biomarkers evaluated. The diagnostic performance of IL6 is equivalent to the combined use of CRP and PCT, in both cases suitable for the identification of patients with sepsis. The addition of IL6 to the biomarkers already used, PCT and CRP, imply a significant improvement and represents the best diagnostic performance. Therefore, we recommend to include IL6 in the diagnostic algorithm of sepsis management in ED because it may assist clinicians in their decision making for early antimicrobial administration, enable risk stratification and expedite the execution of sepsis bundle.

B-073

assessement of the study of intestinal protozoan in the adult chronic diarrhea syndrome

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Background: Stool analysis is of special relevance in the study of adult chronic diarrhea syndrome and should include a microscopic study to assess the existence of leukocytes, blood or fats, in addition to the study of fecal calprotectin. With independence should be studied, infectious, bacterial and parasitic causes. The microscopic study of parasites in stools is a laborious technique that requires experience, and a high time of microscopic observation. Therefore, it is important to know the performance of this test in the context of chronic diarrhea syndrome in adults. The aim of this study is to evaluate the performance of the microscopic study of parasites in adult

patients clinically diagnosed with chronic diarrhea syndrome based on the calculation of post-test probability. **Methods:** A total of 302 adult patients (aged 16-96 years) with clinical presumption of chronic diarrhea syndrome were selected for the pilot study. The microscopic study of the faeces was carried out previous concentration of the sample by the Telemann method. The sensitivity and specificity values of the microscopic technique were calculated based on the data of the work published by *Stensvold et al. J Clin Microbiol 2012*. With these values, the probability coefficient was calculated which together with the pre-test probability (prevalence) were used to calculate the post-test probability. For the calculation of the negative post-test probability and its confidence interval, a methodology based on the Bayes theorem was used in Microsoft Excel based on the calculated prevalence of the disease in our pilot study and the results of the laboratory test. **Results:** In the microscopic study, 8 samples with parasites were detected, equivalent to a pre-test prevalence (prevalence) of 1.98%. All the parasites identified were protozoa: 3 *Endolimax nana* and 5 *Blastocystis hominis*. The negative post-test probability calculated wich Bayes' theorem was 1.7% (CI: 0.4 - 6.5%). **Conclusions:** - Based on the results, 5 out of 100 patients (based on the calculated confidence interval the number of patients would range between 1 and 20) studied for chronic diarrhea and parasitic etiology would not be diagnosed by microscopic techniques. - Therefore, based on the above, for the study of chronic diarrhea syndrome in adults, it would be appropriate to implement a contingent strategy with the use of more sensitive techniques such as molecular diagnosis, thus reducing the number of false negatives in the techniques conventional.

B-074

Mycobacterium tuberculosis: a validation of molecular test for detection of bacteria DNA and resistance to rifampicin

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Background: Tuberculosis (TB) has reached alarming proportions of 10.4 million incidence cases and 1.7 million deaths attributed to the disease as reported by the latest WHO global TB report 2017. In 2016, 66,796 new cases and 12,809 cases of tuberculosis retreatment were registered in Brazil. Globally, some 50 million individuals are already latently infected with multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains creating a remarkable resource for future cases of active TB. The four first-line drugs routinely used in anti-tuberculosis therapy are: isoniazid (INH), Rifampin (RIF), Ethambutol (EMB), Pyrazinamide (PZA). The WHO recognizes the urgent need for more accessible diagnostic tools that are rapid, accurate and associated with detection of resistance to drugs. The GeneXpert MTB/RIF assay (Cepheid's GeneXpert Dx System) was developed to improve TB and RIF resistance detection and to have minimal biological hazards. This system integrates and automates sample processing, nucleic acid amplification, detection of the target sequences using real-time PCR including the rifampin resistance-determining region (RRDR) of *rpoB* gene. **Objective**: Evaluate the performance of GeneXpert MTB/RIF System for molecular detection of *M. tuberculosis* and rpoB gene mutations to rifampicin in Brazilian infected patients in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil). **Methods**: The GeneXpert MTB/RIF assay was performed following manufacturer's instructions. The respiratory specimens samples (sputum) were collected from patients with suspect of TB and they were sent to mycobacteriology diagnostics services on publics health centers (Belo Horizonte, Minas Gerais, Brazil). The results obtained were compared with ours for interlab evaluating. Three repetitions of a specimen were used to determine the intrassay precision and three repetitions of the same specimen in 3 days were used to determine the interassay precision of proposed method. **Results:** A total of 41 patients were processed by GeneXpert MTB/RIF kit. The results showed a concordance between the two centers of 48.7% (20/41) of negative specimens, and 46.3% (19/41) of susceptible specimens and 4.8% (2/41) resistant to RIF. The Kappa index was 0.952 (95% CI= 0.664 to 1.00), indicating almost perfect degree of agreement. A specimen was MTB detected low in public health center but in our laboratory the result obtained was negative MTB. The precision studies presented the same results in all conditions (intrassay and interassay), indicating good reprodubility. **Conclusions**: Our results demonstrate that the Xpert MTB/RIF assay can be used to diagnose of MTB and detection of resistance to RIF with basic laboratory infrastructure. The discordant result due probably the fact that in public center the detection of MTB was next to limit of detection and the result of culture was negative. Besides that the patient is HIV positive and has been treated for MTB for more than 10 years. Tuberculosis still remains a challenge to be overcome in Brazil, even though there are reductions in the incidence and mortality coefficients, the disease is still endemic in the country. The Xpert MTB/RIF assay is a WHO endorsed point-of-care molecular assay able to assess simultaneously diagnosis of MTB and RIF resistance, in approximately 2 hours so becomes a fast and accurate diagnosis.

B-075

Validation of a molecular test for detection and differentiation of Herpes Simplex Virus Type 1 (HSV-1) and 2 (HSV-2) in Institute Hermes Pardini, Brazil.

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Background: Herpes simplex virus 1 (HSV-1) and HSV-2 cause a spectrum of diseases that often present as lesions at oral or anogenital and central nervous system (CNS) sites. According to the World Health Organization (WHO) over half a billion people are estimated to have genital HSV infection globally, and HSV fuels the AIDS epidemic by increasing the risk of HIV acquisition and transmission. Early laboratory confirmation of these infections is performed by viral culture of the cerebrospinal fluid (CSF), or the detection of specific antibodies in serum. The sensitivity of viral culture ranges from 65 to 75%, with a recovery time varying from 3 to 10 days. Serological tests are faster and easy to carry out, but they exhibit cross-reactivity between HSV-1 and HSV-2. Currently, assays based on molecular techniques have been highlighted by clinical laboratories for being more sensitive and specific, and also reduce detection times. **Objective**: To validate a real-time PCR test for the differential detection of these viruses, and to compare it with a Nested-PCR. **Methods**: The samples were obtained from patients with presumptive diagnosis of HSV infection. The types of samples include were cerebrospinal fluid (CSF), whole blood, and genital mucosal samples. The DNA viral was extracted by a silica-based purification (in house method). As positive controls, commercial available viruses were used. For Nested-PCR, the amplicons were visualized in agarose gel electrophoresis. The amplification of real-time PCRs was performed in a 7500 Real-Time PCR System, using the TaqMan detection system with predetermined concentrations of primers and probes. **Results**: A total of 61 samples were examined by qPCR and Nested-PCR. Of these, 45 samples were found to be negative by both tests. The qPCR revealed 16 positive samples: 7 were positive for HSV1; 7 for HSV2 and 2 exhibited coinfection. Twelve samples were positive in Nested-PCR: 4 were positive for HSV1, 7 for HSV2 and 1 coinfection. The qPCR test had a limit of detection (LOD) of 20 copies/µL for HSV1 with a mean Ct value of 34, a standard deviation (SD) of 1.35 cycles, and a coefficient of variation (CV) of 3%. The LOD for HSV2 was 16 copies/µL, with mean Ct value of 29 (SD=1.2; CV=4%). The cross-reactivity test showed negative results when tested against CMV, VZV and EBV in 9 samples. The Kappa coefficient was 0,816 with 95% confidence intervals (CI) of 0,569 to 1.0, indicating nearly perfect agreement between the tests. **Conclusion**: The real-time PCR identified all positive samples detected in Nested-PCR, probably due to the higher sensitivity and not to lower specificity, since the test performance against others virus with potential of cross-reaction was excellent. Furthermore, the data indicated that the qPCR was well validated for the diagnosis of herpes and for the distinction HSV-1 and HSV-2 genome. Since this validation, real-time PCR can be used as part of the diagnostic algorithm of infections caused by these viruses. Accurate HSV detection and typing by molecular methods are considered the methods of choice this improves the diagnosis and guides the specific treatment.

B-076

Performance evaluation of the Beckman Coulter VERIS HBV assay in comparison to the ABBOTT RealTime HBV assay

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Background: The detection and quantification of HBV DNA are essential to diagnose chronic HBV infection, establish the prognosis of related liver disease, and monitor the virologic response to antiviral therapy. The aim of this study was to evaluate the analytical performance of the VERIS HBV assay in comparison to the ABBOTT RealTime HBV assay. **Methods:** Analytical performance of the VERIS HBV assay and method comparison with ABBOTT RealTime HBV assay was assessed according to the CLSI guidelines using 187 plasma samples including 20 drug-resistant HBV. **Results:** The between-day precision ranged from 4.15% for the mean 2.09 log IU/mL to 0.92% for the mean 4.68 log IU/mL. A linear relationship was found over 7 logs for HBV-DNA ($r^2 = 0.9994$; $P < 0.0001$). The lower limit of quantification was estimated at 8.76 IU/mL (95% CI: 7.32 to 12 IU/mL). For Bio-Rad controls, the total CVs were 3.62% (2.30 log U/mL), 2.27% (2.56 log U/mL) and 0.81% (4.38 log U/mL). The Passing-Bablock regression analysis showed good agreement between the VERIS HBV and the ABBOTT RealTime HBV assays in 187 samples ($y = -0.239713 + 0.971264x$), as well as in 20 drug-resistant HBV ($y = -0.541551 + 0.995370x$) samples. The mean differences between the VERIS

and ABBOTT assays were −0.3674 log IU/mL (95% CI, −0.4373 to -0.2974) in 187 samples and −0.44 log IU/mL (-1.40 to 0.51) in 20 drug-resistant HBV. **Conclusion:** The VERIS HBV assay is well-suited to monitoring HBV DNA levels in both chronic HBV and drug-resistant HBV, according to current clinical practice guidelines.

B-077

Prevalence of hepatitis C virus variants resistant to NS5A inhibitors in patients infected with HCV genotype 1b in Southern Taiwan.

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Background:
Hepatitis C virus (HCV) non-structural protein 5A (NS5A) inhibitors have been recently developed to inhibit NS5A activities and have been approved for the treatment of HCV infection. However, the drawback of these direct acting antivirals (DAAs) is the emergence of resistance mutations. The prevalence of such mutations conferring resistance to HCV-NS5A inhibitors before treatment has not been investigated in Chang Gung Memorial Hospital-Kaohsiung Medical Center. The aim of this study was to detect HCV variants resistant to HCV-NS5A inhibitors in hepatitis C patients infected with HCV genotype 1b before any treatment with NS5A inhibitors.
Materials and Methods:
Patients
The current study included 559 patients infected with HCV genotype 1b who were referred to gastroenterology department in our hospital between Dec. 2016 to May 2017.
NS5A amplification
Total RNA was extracted from 1 mL of plasma using Abbott mSample Preparation kit according to the manufacturer's recommendations. The extracted RNA was reverse transcribed using the PrimeScript 1st strand cDNA Synthesis system. First PCR of the HCV NS5A was amplified using the pair of primers as follows: sense 5′-AAGAGGCTCCACCAGTGGAT-3′ and antisense 5′-CGCCGGAGCGTACCTGTGCA-3′. One microliter from the first PCR reaction were used in the nested PCR with the pair of primers as follows: sense 5′-AATGAGGACTGCTCCACGCC-3′ and antisense 5′-GTGAAGAATTCGGGGGCCGG-3'. The nested PCR product obtained was 436 bp in size
NS5A direct sequencing and sequence analysis
The nested PCR products were purification using the DNA Clean & Concentrator according to the manufacturer's instructions. DNA sequencing was performed using the BigDye Terminator v 3.1 Cycle Sequencing Kit with a 3130 genetic analyzer. Nucleotide sequences were aligned with reference sequences AJ238799 for gentoype 1b. The threshold of nucleotide mixture detection during sequencing of sample is estimated to be around 20%.
Result:
The NS5A gene was successfully sequenced in 539 out of 559 (96.4%) samples that were amplified by PCR. Resistance mutation to NS5A region (substitutions of amino acid 28; 30; 31; 58 and 93) were observed in 204/539 (37.8%) sequences analyzed. Y93H (n=90; 16.7%) predominated over P58S (n=37; 6.86%), R30Q (n=21; 3.9%) , L28M (n=8; 1.48%) and L31I (n=4; 0.74%).
Conclusion:
Mutations conferring resistance to HCV NS5A inhibitors are frequent in treatment-naïve patients infected with HCV genotype 1b. Their influence in the context of DAA therapies has not been fully investigated and should be taken into consideration.

B-078

Determination of IgG antibodies to Measles, Mumps, Rubella and Varicella Zoster virus using a fully automated chemiluminescent multiplex analyser system

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Background
Routine detection of Measles, Mumps, Rubella and Varicella Zoster MMRV IgG is used to determine antibody status where infection history or previous immunisation is unknown.
Materials/Methods
This MMRV assay was developed using the Dynex Technologies Multiplier™ fully automated chemiluminescent multiplex analyser system and coated bead technology. Antigen coated beads representing each MMRV specificity were embedded into the base of the wells of the assay plate. Each assay well contains the 4 MMRV targets for the test sample IgG detection. The final chemiluminescent reaction is imaged with the on-board camera and results output as index values referenced against the assay specific calibrator. Precision was measured by assaying a range of 14 samples 3 times across an assay plate on three instruments over three days. A ROC analysis was run in order to set the cut-off for each of MMV and confirm it for Rubella where the cut-off was ultimately defined by the International reference RUBI194. Using the resulting cut-off values, the concordance was assessed on up to 929 samples collected for MMRV screening; results were compared to 510k cleared ELISA assays. Analyse-It® software was used for the ROC analysis and also to generate the 2x2 tables with a Wilson confidence interval set to 0.95%.
Results
Precision
The mean percentage coefficient of variation (%CV) for all four assays varied as follows:
Within run: 3.69-5.35%, between run: 4.06-5.66%, between day: 3.15-4.76% and between instrument: 1.37-3.19%.
ROC analysis
Area under the curve (AUC) and 95% CI results were: Measles 0.995 AUC (0.991-0.998 CI), Mumps 0.987 AUC (0.977-0.997 CI), Rubella 0.998 (0.997-0.999 CI) and VZV 0.999 (0.997-1.000 CI). Percent positive agreement (PPA) and percent negative agreement (PNA) with 95% confidence intervals (CI) were calculated in two ways: Equivocal samples scored as positive
PPA: Measles - 95.3% (93.5-96.6% CI), Mumps: 90.2 (87.8-92.2% CI), Rubella: 93.9 (91.9-95.4% CI), VZV: 98.1 (96.8-98.8% CI). PNA: Measles: 94.2 (90.7-97.0% CI), Mumps: 93.3 (88.5-96.2% CI), Rubella: 99.5% (97.4-99.9% CI), VZV: 97.5 (96.3-99.2% CI).
Equivocal samples scored as negative
PPA:Measles-93.3%(91.2-95.0%CI)Mumps:93.3(91.1-95.0%CI),Rubella:93.0(90.9-94.7% CI), VZV: 97.7 (96.3-98.5% CI). PNA: Measles: 95.4 (92.0-97.4% CI), Mumps: 94.6 (90.8-96.9% CI), Rubella: 100.0 (98.4-100.00% CI), VZV: 99.2 (95.6-99.9% CI).
Conclusion
This multiplexed fully automated assay gives reproducible semi-quantitative results for MMRV IgG. It is ideal for batch testing as it can handle up to ninety two test samples in a single plate to produce 368 results in <3 hours. When two plates are run together, 736 results are generated in 5 hours. *Under development. The performance characteristics of this device have not been established. Not available for sale, and its future availability cannot be guaranteed. The Multiplier is currently Research Use Only*

B-079

Comparison of different molecular assays to diagnose human respiratory viral infections

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Background: Respiratory infections are a major global health problem, mainly affecting young children and the elderly in low- and middle-income countries. The causative agents of this type of infections are viral or bacterial, being viruses more frequently involved. The management of the infections is crucial to prevent epidemics or pandemics, so accurate and specific diagnosis tools are required. The aim of this study is to compare two different Real-Time PCR assays with CLART®PneumoVir kit, which is the hospital routine diagnostic method.
Materials/methods: 108 respiratory samples with a positive diagnosis by CLART®PneumoVir (Genomica) to some of the most common viruses that cause human respiratory infections were included in this prospective comparative study. The samples were collected at Hospital Clínico Universitario Lozano Blesa (Spain) during three years: 2014-2017, comprising different seasonal viruses' subtypes. All samples were analyzed by VIASURE Respiratory Panel (Certest Biotec), FTlyo Respiratory Pathogens 21 (Fast Track Diagnostics, FTD) and CLART®PneumoVir (Genomica). The two first assays are lyophilized ready-to-use Real-Time PCR products whereas the last one is based on reverse transcriptase amplification and visualization in low-density microarray.
Results: The results are shown in the following table:

	VIASURE assay	Fast Track assay	Agreement with reference to CLART®PneumoVir (Genomica)	
	No. positive samples	No. positive samples	VIASURE vs Genomica	FTD vs Genomica
Influenza A/Influenza H1N1/ Influenza B	19	18	100%	94,7%
Coronavirus 229E/ Coronavirus NL63/ Coronavirus OC43	20	18	100%	90,0%
Parainfluenza 2/Parainfluenza 3/Parainfluenza 4	24	4	100%	16,7%
Parainfluenza 1/ Metapneumovirus/Bocavirus/ *Mycoplasma pneumoniae*	21	20	100%	95,2%
Respiratory syncytial virus A/B/Adenovirus	24	22	100%	91,7%

Total: 108 samples

Conclusions: VIASURE Respiratory panel exhibited as good clinical accurancy as CLART®PneumoVir with the additional advantage of being easier to perform and reducing turnaround time. FTD Respiratory Panel was not able to detect some positive samples with the inconvenience of requiring a three-fold increase in the starting amount of RNA template.

B-080

Urine Specimen Stability Comparison in Various Storage Conditions for Sediment Analyses on Atellica UAS 800 Analyzer

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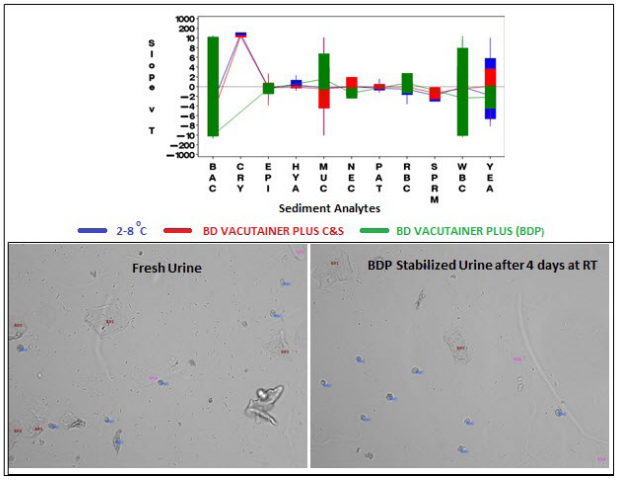
Background: Urinalysis is among most commonly used screening tests in clinical laboratories. Although automatic analyzers reduce analysis time, sample transportation time may lead to delayed preanalytical time. This logistical constrain challenges the laboratories to perform sediment urinalysis within recommended 4 hours. Earlier research reported contradictory stability results with limited number of analytes for up to 72 hours. In this study, the stabilities of 11 urine sediments particles were determined in five storage conditions for up to 96 hours. **Methods:** Urine specimens were received within 4 hours of collection. Several urine specimens were pooled to create 13 pools. Each pool was stored in five conditions: at room temperature, at 2-8°C without preservatives, and at room temperature in BD VACUTAINER PLUS, BD VACUTAINER PLUS C&S Preservative and BORITEX (Aldwin Scientific) urinalysis preservative tubes. The sample-pools (3 aliquots) were analyzed for 4 days in 3 replicates/run on Atellica UAS 800 Analyzer* (Siemens Healthineers). The stability of each analyte was estimated by determining the slope of a regression fit to the recovered sediment particle results as a function of time. The slope of the relationship was considered statistically non-significant if p> 0.05. **Results:** The results indicate room temperature storage caused significant bacterial growth. The results also indicate that the slopes for WBC, RBC, EPI, NEC, BAC, HYA, PAT, YEA, MUC are non-significant at 2-8°C and at room temperature with preservatives for up to 4 days. The results further indicate the CRY slope is significantly positive at 2-8°C; SPRM slope is significantly negative. **Conclusion:** The results suggest that the several urine sediment analytes are stable at 2-8°C without preservative and at room temperature with the preservatives for up to 4 days. CRY and SPRM analytes demonstrated significant instability under all storage conditions evaluated. * Not available for sale in the U.S. Product availability varies by country.



B-081

Prevention of Highly Pathogenic Avian Influenza Virus from Poultry/ Humans and Prediction of Its Outbreak by Satellite

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Background: One third of world population were killed during the 1918 influenza pandemic with half a million deaths only in the United States, as summarized by AACC in 2016. The aim of the present study was to propose the simple and cheap measures for protection of poultry from avian influenza virus (AIV) with cross-species transmission to humans as H5N1, H2N2, H9N2, H7N7. **Methods:** Control and Experiment were compared to see the viral inactivity under measures of chemical, biological, and physical methods in the laboratory in vitro tests of H5N6 and H1N1. **Results:** The present methods showed that the highly pathogenic influenza (HPAI) A virus subtypes have lost the viral activity after treatments of chemical, biological, and physical measures to inactivate the virus. Furthermore, the predictions of place and time of HPAI outbreaks were determined by data of remote sensing satellite from NASA prior to their occurrences in all over the world, including regions without HPAI outbreaks. The initiative results were disclosed on the basis of the incredible linear relationship (R^2 =0.9967) between the year of the AIV outbreaks and the year of minimal average daily sunspot area during 1878 to 2016. **Conclusion:** The source of AIV were penguins in Antarctica and guillemot in Arctica. Migratory birds and humpback whales transmitted low pathogenic avian influenza (LPAI) to the AIV sink of Continents with rice, wheat, maize, waters, and mudfishes persisted and mutated as HPAI under low UV-B exposure, temperature, salinity, relative humidity, and desert dust particles to infect the domestic poultry and humans with HPAI. The present simple measures may save the Earth from HPAI in domestic poultry and humans.

B-082

New sampling strategies to detect environme ntal microbial contamination and to verify disinfection and sterilization procedures

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Background: Hospital-acquired infection are often connected to contamination of inanimate surfaces near the patients. Up to day, there is not standardize and efficient methods to evaluate the microbial contamination and consequently assess the efficacy of the cleaning procedures. The sampling of the surfaces can be performed using contact plates or swabs. Contact plates are used for sampling of flat

surfaces. Swabs are used for sampling of articulated surface. Aim of the study was to investigate a new device for surface monitoring, the FLOQSwab in combination to SRK solution (Hygiene Monitoring System HMS, Copan Italia) to evaluate the efficacy of the sanitization method used to clean surfaces in Hospital wards **Methods:** The following Hospital wards were considered for the monitoring: Dialysis Center (n=5 sampling points); Gynecology Surgery Room (n=14 sampling points) and Orthopedic Radiology (n=5 sampling points). Cleaning procedure: identified sampling points were cleaned using a disinfection system (HyperDRYMist® technology). Sampling was performed in parallel before and after the cleaning procedure with a new device and the traditional swab. To standardize the area to be sampled, a square cardboard frame 10 x 10 cm (COPAN Italia) was used to define the area for testing. The flocked swab was transferred in its transport medium tube (1mL of SRK solution) and the traditional swab in 1ml of saline solution. The whole 1 ml was used to inoculate Tryptic Soy Agar plate at 35°C up to 3 days. The bacteria identification was performed by mass-spectrometry **Results:**The efficacy of the sanitization procedure was evaluated on the difference in colonies count detected on the surface before and after sanitization. In all wards considered, the use of HMD has allowed to identify more bacteria species then the traditional swab. In all the sampling points, HMD was able to detect on the different surfaces the "thrue microbial load", the rayon swab reported an underestimation of the microbial load in all analyzed sampling sites.**Conclusion:**Use of Nylon Flocked swabs as improved swab sampling device and SRK solution as preservation medium allowed to adequately assess the microbial contamination on the surfaces sampled and thus properly evaluate the effectiveness of disinfection system used.

B-083

In vitro starvation model for Assessing Phenotypic Drug Tolerance on Mycobacterium Tuberculosis Lineages in Ethiopia

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Abstract

Background: Mycobacterium tuberculosis persist in the human host for decades& reactivation can occur at any point. Becomes dormant and phenotypically drug tolerant when exposed adverse conditions. Understanding of the signals and processes which allow the bacteria to achieve this feat could potentially be used as a baseline to design new types of drugs or modify old drug regimens for improved cure and avert development of drug resistance. **Objective:** To use *in vitro* starvation model in assessing if nutrient deprivation affects phenotypic drug tolerance in *Mycobacterium tuberculosis* lineages circulating in Ethiopia. **Methods:** Three MTB lineages and one standard susceptible reference strain (H37Rv) were tested by different test methods at different time point from March to September 2017. All lineages tested to be sensitive to first line anti Tb drugs. Log phase (highest colony count on week 3-4) culture from Lowenstein Jenson medium sub cultured to Middle-brook7H9 with 10% Oleic Acid Albumin Dextrose Catalase as a normal, Phosphate Buffer Solution (PBS) (PH 7.2) and Sterile Distilled water (SDW) as starvation media were used. Each week we performed culture growth reading, Acid Fast Stain (AFS) by Zeihel Nelson (ZN), Lipid Bodies (LB)by Sudan black stain and viability by Fluorecin DiAcitate (FDA) staining. On week 0, 3 and 6 drug susceptibility test was done by colorimetric MTT assay. Graph pad prism 6 and SPSS V20 used for data analysis. **Results**: A total of 576experiments were performed using4 strains of *Mycobacterium Tuberculosis*subcultured on SDW, PBS and 7H9 and. Of these, 324 microscopic tests using (108(ZN) acid fastness, 108(FDA) viability, and 108(Sudan black stain) lipid bodies), 108 culture growth reading done. After week 6acid fastness, viability and culture growth decreased. 144 phenotypic DST done using MTT assay. A higher inhibitory drug concentration was required at the 6th week compared to the baseline and C50 (RMP=0.5; INH=0.1; STM=2.0 and for EMB=4.0), yet the proportion of lipid body containing bacilli increased continuously in all lineages. **Conclusion**: Our study showed that the mycobacteria lineages behaved similarly in all media systems and reached stationary phase at similar time. The increased drug concentration observed at the 6th week coincided with the decline in viable bacilli in all media systems, thus attributing this phenomena to lipid body accumulation alone was difficult.

Keywords: M. tuberculosis, LB%, Drug Tolerance, and MTT Assay.

1

B-084

Performance Evaluation of the VITROS® Immunodiagnostic Products B·R·A·H·M·S PCT Assay on the VITROS 3600 Immunodiagnostic Systems

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Background: We have evaluated the performance of VITROS® Immunodiagnostic Products B·R·A·H·M·S PCT (Procalcitonin) assay (in development), which consists of VITROS® B·R·A·H·M·S PCT Reagent Pack and the VITROS® B·R·A·H·M·S PCT Calibrators on the VITROS® 3600 Immunodiagnostic Systems using Intellicheck® Technology. **Methods:** The VITROS® B·R·A·H·M·S PCT assay is a two-step dual monoclonal immunometric assay that uses anti-PCT antibody immobilized on the well surface to capture PCT in the patient sample. Unbound PCT in the sample is removed by washing and the detector antibody (anti-PCT Mab) horseradish peroxidase (HRP)-labelled conjugate is added. Unbound HRP conjugate is removed by a second wash and the bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrate (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the System. The amount of HRP conjugate bound is directly proportional to the concentration of PCT present in the sample. The time to first result in the system is 24 minutes. **Results:** The assay is calibrated against the B·R·A·H·M·S PCT™ sensitive KRYPTOR™. Limit of quantitation was determined to be 0.006 ng/mL. Linear regression analysis showed linearity across the range of 0.011 to 95.58 ng/mL. Precision study over 22 days with five precision pools showed excellent precision with sample concentrations of 0.040 ng/mL, 0.429 ng/mL, 1.69 ng/mL, 8.63 ng/mL, and 46.38 ng/mL resulting in within-laboratory percent coefficient of variation (%CV) of 7.5%, 3.0%, 3.0%, 5.0%, and 3.4% respectively. Patient samples showed acceptable results up to 20-fold dilution. No evidence of high dose hook was observed up to 5,000 ng/mL. The accuracy of the VITROS® B·R·A·H·M·S PCT assay was evaluated with 210 patient specimens (range: 0.11 to 93.38 ng/mL) against the B·R·A·H·M·S PCT sensitive KRYPTOR. The following regression statistics using Passing and Bablock was obtained: VITROS PCT = 0.98* B·R·A·H·M·S PCT sensitive KRYPTOR- 0.04; Pearson Correlation Coefficient (r) = 0.98. No significant interference or cross-reactivity were observed with biotin (3,500 ng/mL), conjugated bilirubin (32.3 mg/dL), unconjugated bilirubin (47.6 mg/dL), hemoglobin (500 mg/dL), heparin (8000 IU/L), total protein (1.65 g/dL), triglycerides (17.76 mg/mL), HAMA (>160 IU/mL), and RF (282 IU/mL). The samples can be stored up to 24 hours at room temperature, 48 hours refrigerated and up to three freeze-thaw cycles. Serum, EDTA and lithium heparin matrices showed acceptable results. The reference range using negative samples based on central 95th percentile was 0.004 ng/mL to 0.037 ng/mL. The VITROS B·R·A·H·M·S PCT assay showed excellent negative and positive percent agreements compared at the B·R·A·H·M·S PCT sensitive KRYPTOR at medical decision cutoff of 0.10 ng/mL, 0.25 ng/mL, 0.50 ng/mL and 2.0 ng/mL.**Conclusion:** In summary, the VITROS® B·R·A·H·M·S PCT assay demonstrates reliable and acceptable performance on the VITROS 3600 Immunodiagnostic Systems.

B-085

Active Surveillance Cultures: Frequency of Microorganisms and Phenotypic Resistance Profile from Public Hospitals of Sao Paulo City, Brazil

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Background: Surveillance cultures are routinely used by public health authorities to screen for multidrug resistant bacteria. In the past few decades, the widespread use of broad spectrum antibiotics has provided the acquisition of resistance genes that in general are carried by plasmids, which contribute to rapid spread of resistance genes within the bacterial population. The purpose of this study was to determine the prevalence of multidrug-resistant (MDR) organisms isolated from rectal swab screening in several Brazilian public hospitals represented by the north, south, east and west regions of the São Paulo city. **Methods:** Surveillance samples were analyzed from 1st January to 30st December 2017. All the rectal swab samples were collected from patients admitted from high-risk settings or transferred from areas with high rates of MDR organisms. All clinical specimens were inoculated onto a selective media (ChromID media, bioMerieux) and incu-

bated at 35±2°C for 18-24 h. The screening test was performed to Gram-negative bacilli (GNB) using the antibiotics ertapenem, imipenem and meropenem and for Gram-positive Cocci (GPC) screening was tested oxacillin (methicillin) and vancomycin using disc diffusion method. Bacteria identification was performed by MALDI-TOF mass spectrometry (Vitek-MS, bioMerieux) and, as required, the minimal inhibitory concentrations (MIC) of antibiotics were determined using the Vitek 2 System (bioMerieux). **Results:** A total of 22.641 rectal swab samples were processed. 3.084/22.641 (13.62%) showed positive results for the presence of microorganisms. Out of which, 2.344 (76%) were Gram-negative Bacilli (GNB) and 740 (24%) were Gram-positive Cocci (GPC). The majority of the isolated carbapenems resistance was *Klebsiella* spp. [1.872/3.084 (60.7%)], which the *Klebsiella pneumoniae* was the predominant species, followed by *Acinetobacter baumannii* [215/3.084 (7%)], *Pseudomonas aeruginosa* [125/3.084 (4%)], *Enterobacter* spp. [102/3.084 (0.97%)] and others BGN [30/3.084 (0.1%)]. Between the GPC the resistance to vancomycin was observed mainly in 16.8% *Enterococcus faecalis* (519/3.084) and 7.1% *Enterecoccus faecium* (220/3084). **Conclusion:** The findings in this study corroborates with other Brazilian studies, which *K. pneumoniae* carbapenem resistant was the most frequent organism recovered from rectal swab samples followed by *Enterococcus* spp. vancomycin resistant. Early colonization detection by screening assays should be used to minimize the chance of transmitting MDR organisms from colonized to non-colonized patients, reinforce the continued need for infection control hospital surveillance system.

B-086

Performance of Bio-Rad Laboratories HIV Quality Controls on the VITROSâ Immunodiagnostic Products HIV Combo assay.

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Background: Even with advanced blood screening and therapeutic options, HIV contamination or infection remains a serious threat to the world's blood supply and a major health issue for at risk populations. Constant vigilance, in the form of diligent screening of donated blood and accurate diagnosis in potentially infected people, is necessary to prevent a resurgence of this disease. Organizations such as the US Centers for Disease Control (CDC) and the World Health Organization (WHO) have defined very rigorous regimes for HIV testing, which include screening and confirmatory assays. An integral part of this testing regime is the use of third party quality controls, which confirm the reliability of these important tests and allow for a large measure of confidence in the reported results. **Objective:** The performance of five Bio-Rad HIV Quality Controls on the recently introduced Ortho Clinical Diagnostics VITROS HIV Combo 4th generation assay was examined. The controls (and analytes) were as follows: VIROTROL I (anti-HIV-1), VIROTROL HIV-2 (anti-HIV-2), VIROTROL HIV-1 Ag (HIV-1 Ag), VIROTROL HIV-1 gO (anti-HIV-1 gO) and VIROCLEAR (negative control). VIROTROL HIV-1 gO is not intended for use with blood screening assays. The VITROS™ HIV Combo Assay is for the simultaneous qualitative detection of antibodies to HIV-1 (including group M and O) HIV-2 as well as HIV p24 antigen in human serum and plasma using a chemiluminescent immunoassay (ChLIA) methodology. The VITROS HIV Combo assay is intended for diagnostic purposes only. The effectiveness of the VITROS HIV Combo assay for blood and/or plasma screening has not been established in the United States. **Method:** The evaluation of the Bio-Rad Laboratories HIV Quality Controls was performed using three different lots of the VITROS™ HIV Combo assay and utilized the VITROS™ ECi/ECiQ, 3600 or 5600 immunodiagnostic systems. The thirty week study was performed at three sites resulting in a total of 214 data points for each control (and analyte). Unopened samples of each Bio-Rad control were tested either daily or at approximately four week intervals, depending on the test site. **Results:** Test results for the controls were 100% in agreement with the expected "reactive" or "non-reactive" result, depending on the intended use of the quality control. **Conclusion:** Bio-Rad VIROTROL and VIROCLEAR controls are optimally suited for use as third party quality control materials on the Ortho Clinical Diagnostics VITROS HIV Combo assay, ensuring confidence in overall test performance and reported patient diagnostic test results.

B-087

Comparison of Abbott Architect Syphilis TP test and Bio-Rad Syphilis IgG test on BioPlex 2200.

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Background: Implementation of Beckman Automation Line (Power-Express) with directly attached Abbott Architect instruments (one of the first in the USA) prompted us to compare 2 methods of reverse algorithm syphilis testing between Bio-Rad Syphilis IgG kit on BioPlex 2200 (used in our lab for last few years) with Abbott Syphilis TP testing on Architect (qualitative detection of antibodies IgG and IgM directed against Treponema Palidum). **Methods:** Consecutive 1007 patients samples were tested in both systems. According to our policies, all positive or equivocal samples on BioPlex instrument were followed with RPR and TPPA testing. Additionally all positive samples on Architect had PRP and TPPA performed regardless of BioPlex results. **Results:** From the pool of 1007 patients, 857 had negative and 137 had positive results on both instruments(Cohen's kappa agreement 94,8 %).From 5 equivocal samples on BioPlex 3 were non-reactive and 2 reactive on Architect, RPR and PTTA.There were 5 reactive samples on BioPlex which were non- reactive on Architect, RPR and TPPA. There were 2 reactive samples on Architect which were non-reactive on BioPlex, RPR and TPPA.One sample reactive on Architect was non-reactive on BioPlex but reactive for RPR and TPPA. **Conclusion:** Our study confirms good agreement between these 2 methods of reverse algorithm syphilis testing.Minimal differences between these methods could be partially explain by design of the tests with equivocal zone on BioPlex 2200 and additional detection of IgM antibodies in Architect test.

B-088

Integrating exosomal microRNA and electronic health data to promote tuberculosis diagnosis

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Background: Tuberculosis (TB) is difficult to diagnose from complex clinical conditions. Diagnostic information from electronic health records (EHR) remains insufficient. Currently, exosomal miRNAs are emerging as biomarkers for diseases. We aim to investigate the potential of exosomal miRNAs and EHR in TB clinical diagnosis. **Methods:** 388 individuals were interrogated with a prospective multi-stage approach. Exosomal miRNA expressions were profiled with microarray followed by qRT-PCR. EHR and follow-up information of patients were collected accordingly. In discovery phase, differentially expressed miRNAs (DEM) were narrowed down and further selected. In selection and testing phases, models with 'EHR + miRNA' and 'EHR only' were established using support vector machine. We relieved the overfitting problem with unsupervised approach, model interpretation and testing phase. We in silico predicted the targeted genes of DEM, networks of DEM with related GO or KEGG pathways. **Results:** 351 individuals were finally enrolled. Six DEM (20a, 20b, 26a, 106a, 191, and 486) were over-expressed in pulmonary tuberculosis (PTB) and tuberculous meningitis (TBM) patients as compared with their controls. 'EHR + miRNA' model showed a better diagnostic efficacy for TBM than 'EHR only' model (AUC: 0.87 vs 0.70, sensitivity: 0.83 vs 0.71, specificity: both 1). Modelling with or without miRNAs both achieved satisfactory performance for PTB. DEM presented a decreased trend after 2-month intensive therapy (adjusted-p = 4.80 ×10-5). DEM were predicted to involve in immunologically regulation and neurotrophin receptor signaling. **Conclusion:** Our present study identified 6 exosomal miRNAs as promising noninvasive biomarkers for PTB and TBM patients. Combination of exosomal miRNAs and EHR through machine learning algorithm could serve as a feasible approach in promoting TBM differential diagnosis, and further prospective validation is required before its clinical utility.

B-089

Novel rapid quantification method of bacteria in a septic blood sample can produce an effective biomarker for monitoring patient care

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Background: Severe systemic infections, such as sepsis, are the primary cause of morbidity and mortality in hospitalized patients. Current biomarkers in sepsis do not always reflect the severity of sepsis at a particular point in time. Acquiring the earliest possible identification of pathogenic microorganisms is critical for selecting the appropriate antimicrobial therapy and obtaining a favorable outcome in infected patients. Here we developed a novel rapid identification and quantification method of unknown pathogenic bacteria in a clinical sample, and estimated the usability of blood bacterial concentration as a novel biomarker in sepsis. **Methods:** We have already reported the development of a rapid diagnostic method, called the Tm mapping method, which requires neither microbial cultures nor DNA sequencing to identify the causative pathogenic bacteria. This method is based on real-time PCR with seven primer sets, and the algorithm generates a unique "finger-print" of the bacterial species from the data of the melting temperature (*Tm*) of each PCR amplicon. This "finger-print" is compared with those of more than 150 bacterial species in the database. The software and database is accessible by Internet, and the output is the list of the bacterial species in the order of the matching score, called Difference Value. As a result, we can get an identification result of pathogenic bacteria around four hours after whole blood collection. In this research, we tried to improve the Tm mapping method to not only identify but also quantify bacteria in a sample. **Results:** We identified and quantified pathogenic bacteria in 26 septic blood samples, and the blood bacterial concentrations were correlated with the severity of sepsis (qSOFA, septic shock, Pitt Bacteremia Score). We subsequently examined the time-dependent changes (pretreatment, and 24 to 72 hours after antibiotic treatments) of blood bacterial concentration, and found that the time-dependent changes of blood bacterial concentration were dramatically decreased compared with the change of Body temperature (BT), White blood cells (WBC), C-reactive protein (CRP), Procalcitonin (PCT), Presepsin (P-SEP) and Interleukin-6 (IL-6). **Conclusion:** We developed a novel rapid identification and quantification method of unknown pathogenic bacteria in a whole blood sample, and found that the blood bacterial concentration would be useful as a novel biomarker not only to estimate the severity of sepsis but to monitor the therapeutic effect.

B-090

One-Step Real-Time PCR assay using a novel primers-probe set for universal detection of Dengue virus

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Background: Dengue virus (DENV) infection is the most important arthropod-borne viral infection of humans and the incidence of dengue has grown dramatically. According to WHO is estimated there are up to 390 million DENV infections annually, with more than 500,000 hospitalizations and 25,000 deaths. The Dengue virus group consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that manifest a diverse range of symptoms. Given that dengue virus infection elicits such a broad range of clinical symptoms, early and accurate laboratory diagnosis is essential for appropriate patient management. Molecular methods such as RT-qPCR have become a primary tool to detect virus in the early course of illness. In addition, molecular testing allows provide same- or next-day diagnosis of DENV during the acute phase of disease, thus permitting the monitoring of outbreaks and the implementation of control measures. **Objective**: To describe the validation of a one-step real-time PCR (RT-qPCR) using a novel universal sets of primers and hybridization probes for detection of dengue virus serotypes 1-4 in serum samples. **Methods**: The primers and probe were designed using the Primer Explorer V4 software. To assure the specificity of the primers, the 3' untranslated region of all complete genome sequences of dengue virus was selected and downloaded from GenBank, and aligned with multiple sequence alignment tools to identify the conserved region. Performance of one-step real-time PCR was evaluated using commercial controls. The ability of the assay to detect DENV in clinical samples was tested in 14 serum samples obtained from patients who had presented with dengue-compatible symptomatology and were confirmed to be DENV positive by standard laboratory diagnosis (Nested PCR). The amplification efficiencies and detection limits of this assay were determined. **Results**: A BLAST search against all available sequence databases at NCBI and an in silico PCR did not identify any additional homologous sequences, suggesting adequate performance and high specificity of the designed primers. The detection limits of the studied assay were 30 copies/reaction for DENV-1 and DENV-3 and 15 and 60 copies/reaction for DENV-2 and DENV-4, respectively. The regression equations obtained show good amplification conditions with positive correlation between the variables, with a coefficient of determination (r^2) varying from 0.86 to 0.98. The results obtained with clinical samples showed that 12 samples were positive for DENV and that the assay did not cross-react with other human pathogenic flaviviruses. **Conclusion**: The results suggest that this primer-probe combination could be the basis for development of new real-time PCR assay for laboratory diagnosis of dengue infection. In this method the reverse transcription and PCR processes are conducted consecutively on a real time PCR system. The rapid detection of the DENV by one-step real-time RT-qPCR has become a trend in diagnostic medicine. The proposed assay is efficient, sensitive, specific and less labor-intensive compared to the nested PCR. Advances in molecular methods have improved the sensitivity and specificity of diagnosis of dengue virus infection. It is expected that the application of these assays will contribute significantly to the clinical treatment, etiologic investigation, and control of this infection

B-091

Elevation of D-dimer is linked to disease severity and predicts fatal outcomes in H7N9 infection

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Background: To assess whether an increased D-dimer levels was related to worse global, renal, heart, and respiratory outcomes in critically ill patients with H7N9 infection and whether D-dimer could serve as a biomarker of severity. **Methods:** D-dimer levels in Plasma were serially measured on day 1, day 7, day 14 and day 21 of admission for 130 H7N9 patients (45 lethal and 85 non-lethal cases). 79 H1N1 patients and 71 healthy volunteers were selected as controls. To assess clinical illness severity, both APACHEII scores and the Pneumonia Severity Index (Pneumonia Severity Index class) were calculated. **Results:** Plasma D-dimer level in H7N9 patients was significantly higher than those in H1N1 patients and normal controls (P <0.001).The plasma D-dimer level in death group was significantly higher than that in survival group (P <0.001). Plasma D-dimer level in survival group was significantly lower than that on day 1 (P <0.001). Plasma D-dimer level in death group increased sharply from the day 7 to the day 14 after admission, and decreased significantly from the day 14 to day 21, with statistically significance (P <0.05). Plasma D-dimer levelswere positively correlated with hypersensitive C-reactive protein (HsCRP) and procalcitonin (PCT), liver indicators(ALT and AST)and cardiac indicators (CK, CKMB, LDH), as well as severity indicators PSI and APACHEII scores (r = 0.408 and 0.325, P <0.001). The area under the ROC curve for prediction of patient death at a plasma D-dimer level of 3943 ug/L FEU was 0.811, with a sensitivity of 81.6% and a specificity of 73.8%, better than HsCRP and PCT. The survival rate of the groupof patients with D-dimer> 3943 ug/L FEU was significantly lower than that of patients with D-dimer≤3943 ug / L FEU (P = 0.024). **Conclusion:** Plasma D-dimer levels have certain correlation with the severity and prognosis of H7N9 avian influenza. The higher the plasma D-dimer level, the lower the survival rate of H7N9 patients. Monitoring D-dimer levels can help physicians to determine the severity and prognosis of H7N9 avian influenza. **Financial support:** This work was supported by the China NationalMega-Projects for Infectious Diseases (grant number 2017ZX10103008); and the National Natural Science Foundationof China (grant numbers 81672014 and 81702079).

B-092

Neutropenia has a limited effect on plasma calprotectin levels

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Background: Antibiotics resistance is a growing problem worldwide and there is a need for better markers for bacterial infections to be able to distinguish between bacterial and viral infections. Plasma calprotectin may be an interesting early marker for bacterial infections. Calprotectin is manly expressed in neutrophil granulocytes, but is also found in macrophages and monocytes. A potential problem could be that calprotectin cannot be used in patients with low neutrophil counts or in case when neutrophils are attracted to the site of inflammation and are not present in the circulation. The aim of this study was to evaluate the association between neutrophil counts and plasma calprotectin levels.**Methods:** The study was performed

at Uppsala University Hospital. Plasma calprotectin was measured in Li-heparin plasma on a Mindray™ BS-380 (Mindray Medical International, Shenzhen, China) with reagents from Gentian (Moss, Norway). The instrument settings for the method were: sample volume=3 µL, R1 volume=200 µL and the R2 volume=30 µL. The wavelength was 605 nm and the total assay time was approximately 10 min. The calprotectin values in the study cohort varied between 0.09 and 33.1 mg calprotectin/L. The patients had neutrophil counts in the range <0.1-16.1 x 10^9/L. The calprotectin levels in the samples (n=56) were correlated with the neutrophil counts. **Results:** There was a very weak association between the neutrophil counts and calprotectin levels ($y = 0.28x + 1.25$, $R^2 = 0.037$). Even patients with neutrophil values <0.1 x 10^9/L had detectable calprotectin levels. **Conclusion:** In our study neutropenia had a limited effect on calprotectin levels. The results indicate that calprotectin could also be used in patients with low neutrophil values. Further studies are needed to study the use of plasma calprotectin in different patient populations.

B-093

Turnaround time of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

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Background: Xpert MTB/RIF assay (Xpert) has the potential to rapidly diagnose pulmonary tuberculosis. The purpose of this study was to evaluate turnaround time (TAT) of Xpert during routine clinical use in an intermediate burden setting. **Methods:** Between July 2014 and December 2016, a total of 2,952 consecutive respiratory specimens were simultaneously tested by Xpert, mycobacterial culture, and smear microscopy. **Results:** Compared with smear microscopy, the median TAT of Xpert was significantly shorter (median [interquartile range, IQR] 3.1 [2.3-5.6] hr versus 19.1 [6.8-21.9] hr, $P < 0.0001$). When the time limits were stratified within 3, 6, 12, and 24 hours, the cumulative TAT compliance rates of Xpert were significantly higher compared with smear microscopy (within 3 hours, 49.1% [1,450/2,952] versus 0.4% [13/2,952], $P < 0.0001$; within 6 hours, 76.8% [2,267/2,952] versus 16.7% [492/2,952] $P < 0.0001$; within 12 hours, 80.5% [2,375/2,952] versus 41.4% [1,222/2,952] $P < 0.0001$; within 24 hours, 96.3% [2,842/2,952] versus 88.7% [2,619/2,952], $P < 0.05$, respectively). Bland-Altman analysis for TAT differences of individual specimens between Xpert and smear microscopy showed that Xpert had faster TATs than smear microscopy in 94.5% (2,791/2,952) of specimens. Moreover, the addition of one Xpert module significantly shortened the mean TAT from 3.7 hours (2.5-6.4 by 1 module) to 2.6 hours (2.1-4.6 by 2 modules) ($P < 0.0001$). **Conclusion:** The median TATs of Xpert were remarkably shorter than those of smear microscopy. Moreover, Xpert displayed a higher TAT compliance rate within 24 hours than smear microscopy. Collectively, our findings suggest that the ability for Xpert to rapidly report results may have a clinically profound impact on tuberculosis treatment initiation in an intermediate tuberculosis-burden setting.

B-094

Performance Evaluation of the Atellica IM HBsAgII (Qualitative), Atellica IM HIV Ag/Ab Combo (CHIV) §, and Atellica IM aHCV§ Assays at Two Hospital Sites

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Background: We evaluated recently introduced automated immunoassay analyzer Atellica IM 1600 (Siemens Healthineers, NY, USA) for detecting serologic Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), and Hepatitis B surface Antigen (HBsAg) markers by comparison with the results obtained from ARCHITECT i4000SR (Abbott Diagnostics, Abbott Park, IL, USA). **Methods:** For each HCV, HIV, and HBsAg study, over 1000 hospital routine samples prospectively assayed on Abbott ARCHITECT (both negative and positive for HCV, HBsAg and HIV-1 group M and HIV-2) were then tested on the Atellica IM 1600 Analyzer. The diagnosis of positive results was based on relevant marker profile and clinical and serological data available. For all the discordant results with ARCHITECT, samples were repeated on both methods. If discordant results remained, when possible, further testing was performed: nucleic acid testing and Siemens Healthineers R&D for HCV, confirmatory tests for HIV, and neutralization testing for HBV. Precision for the Atellica IM Analyzer assays was performed according to CLSI EP15-A3: Samples comprised Atellica IM HCV, CHIV, and HBsAg positive QC, BIORAD QC, and a plasma pool at a concentration close to the cutoff – one run per day, five replicates per run, for five days, for a total of 25 replicates per sample. Agreement was calculated vs. respective ARCHITECT assays. **Results:** Precision studies agreed with the manufacturer's claims. Preliminary concordance for HCV was 98.5%; for HBsAg was 99.8%; and HIV 99.9%. Discordant samples are under investigation and final sensitivity and specificity will be calculated. **Conclusions:** The Atellica IM HCV, CHIV, and HBsAgII assays demonstrated acceptable precision on the Atellica IM Analyzer, and good agreement with the Abbott Architect HCV, HIV Ag/Ab Combo, and HBsAg assays even though discordant samples require further investigation.

Precision assays according to CLSI EP15-A3			
Atellica IM Analyzer Assay	**Mean index**	**Within run %CV(SD)**	**Within lab (total) %CV(SD)**
HCV	0.10	4.5(0.0)	4.9(0.01)
	1.25	1.9(0.02)	3.7(0.05)
	3.28	2.6(0.08)	3.1(0.10)
	4.34	2.7(0.12)	3.6(0.16)
CHIV	0.16	8.3(0.01)	8.3(0.01)
	0.96	1.4(0.01)	3.3(0.03)
	3.55	1.7(0.06)	2.7(0.10)
	4.75	1.9(0.09)	3.2(0.15)
	4.89	1.3(0.06)	3.0(0.15)
	6.09	2.1(0.13)	2.6(0.16)
HBsAg	0.12	13.9(0.02)	14.0(0.02)
	0.80	5.1(0.04)	5.1(0.04)
	5.80	2.7(0.16)	2.7(0.16)
	5.83, 9.93	1.8(0.10), 1.9(0.18)	1.9(0.11) 2.5(0.24)

B-095

Use of BACTEC MGIT 960 System to growth for Mycobacteria from clinical specimens in association of Public Hospitals Northern Anatolian Region of Istanbul

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Background: Tuberculosis continues to be a major health problem worldwide and also our country. Rapid and accurate diagnosis is key to controlling the disease. The traditional tests for TB produce results that are either in accurate or take too long to be definitive. Recent advances in new techniques have shortened the time needed to diagnose tuberculosis, leading to improved case detection and management; however, culture is still essential for drug susceptibility testing and improve the diagnostic yield for specimens.In this study it was aimed to determine the diagnosis of *Mycobacterium tuberculosis* infection rates at the patients followed by tuberculosis suspected in the hospitals where we serve, and compare the performance of the BACTEC MGIT 960 in fully automatic system with Lowenstein-Jensen medium.**Methods:** A total of 5548 specimens obtained from 2978 patients were cultured in parallel. Whose cultures were retrospectively evaluated from January 2017 to December 2017 from 13 hospitals at the the Central Tuberculosis Laboratory of Istanbul Northern Anatolian Association of Public Hospitals. **Results:** Of the 5548 specimens included in the study obtained from 2978 patients were cultured. 91% of diagnostic cultures turned positive within 14 days. 79% of them being represented by M.tuberculosis complex. The best yield was obtained with the BACTEC MGIT 960 (Beckton-Dickinson, USA) system with 405 isolates. To comparison with 405 isolates with the BACTEC MGIT 960 system, 374 isolates obtained with Lowenstein-Jensen medium in parallel cultures. The shortest times to detection were obtained with the BACTEC MGIT 960 system (10.7 days average); 14 days earlier than that with Lowenstein-Jensen medium (24.7 days average) . The BACTEC MGIT 960 system

had a contamination rate of 7%, Lowenstein-Jensen medium 12%. The best yield was obtained with the BACTEC 960 system, with 405 isolates, in comparison with 405 isolates with the BACTEC MGIT 960 system and 374 isolates with LJ medium. **Conclusion:** BACTEC MGIT 960 system is a fully automated, nonradiometric instrument that is suitable for the detection of growth of tuberculosis and other mycobacterial species and that is characterized by detection times that are even shorter than LJ medium. A fast and reliable diagnostic method that would differentiate between active and latent TB infection is also lacking.

B-096

Use machine learning-based approach to analyze MALDI-TOF MS data for a rapid and accurate reporting MRSA

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Background: Early discriminating Methicillin resistant *Staphylococcus aureus* (MRSA) from methicillin sensitive *Staphylococcus aureus* (MSSA) could direct correct antibiotics administration. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) may provide early report of antibiotics susceptibility than conventional method. However, detecting antibiotics resistance by using massive data of MALDI-TOF MS has not been widely validated yet. A machine learning (ML)-based approach could serve as a potential tool in analyzing MALDI-TOF MS data for a rapid and accurate reporting MRSA. **Methods:** Two cohorts of *S. aureus* isolates were consecutively collected from clinical specimens in two distinct teaching hospitals. The isolates were analyzed by MALDI-TOF MS to obtain mass spectra. Determination of MSSA or MRSA was performed by disc diffusion. For applying ML, binning method was used first to standardize the peaks of mass spectra. Two feature selection methods, Pearson correlation coefficient (PCC) and One Rule were applied for selecting robust peaks. Various ML algorithms, namely support vector machine, k-nearest neighbor, decision tree (J48), and Random Forest were trained by the training cohort. The performance was externally validated by the test cohort. **Results:** The training cohort contained 3990 cases (MRSA: 2017; MSSA: 1883), while the test cohort was composed of 2100 cases (MRSA: 972; MSSA: 1128) cases. The error window of binning method was set with 10 m/z for standardizing the peaks. To design the prediction models, 43 peaks were selected by PCC. Among the various ML algorithms, J48 model outperformed the others, exhibiting 77.92% accuracy, 74.8 sensitivity, and 81.3% specificity in distinguishing MRSA from MSSA. **Conclusion:** A rapid and accurate preliminary report of MRSA could be accomplished by using the ML-based methodology. Early administration of correct antibiotics against *S. aureus* may have benefit in preventing morbidity, mortality, and shorting length of stay.

B-097

Decreased Siglec-9 expression on natural killer cell subsetassociated with persistent HBV replication

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Background: Siglec-9 is a MHC-independent inhibitory receptor selectively expressed on $CD56^{dim}$ NK cells. Its role in infection diseases has not been investigated yet. Here we studied the association of NK Siglec-9 with chronic hepatitis B (CHB) infection. **Methods:** Flow cytometry evaluated the expression of Siglec-9 and other receptors on peripheral NK cells. Immunofluorescence staining was used to detect Siglec-9 ligands on liver biopsy tissues and cultured hepatocyte cell lines. Siglec-9 blocking assay was carried out and cytokine synthesis and CD107a degranulation was detected by flow cytometry. **Results:** Compared to healthy donors, CHB patients had decreased Siglec-9^{+} NK cells, which reversely correlated with serum HBeAg and HBV DNA titer. Siglec-9 expression on NK cells from patients achieving SVR (sustained virological response) recovered to the level of normal donors. Neutralization of Siglec-9 restored cytokine synthesis and degranulation of NK cells from CHB patients. Immunofluorescence staining showed increased expression of Siglec-9 ligands in liver biopsy tissues from CHB patients and in hepatocyte cell lines infected with HBV or stimulated with inflammatory cytokines (IL-6 or TGF-β). **Conclusion:** These findings identify Siglec-9 as a negative regulator for NK cells contributing to HBV persistence and the intervention of Siglec-9 signaling might be of potentially translational significance.

B-098

Implementation of an Infectious Disease Cloud Based Epidemiology Network in the United States and South America

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Background: Real-time data collection of respiratory disease is important for understanding the spatiotemporal dynamics of disease transmission worldwide. United States (US) healthcare professionals use tools such as FluView to help identify local pathogen circulation; however, these tools are limited to syndromic surveillance, track a limited set of pathogens and do not typically span multiple continents. Understanding respiratory disease dynamics is facilitated by 1) a large, pathogen rich data set 2) geographically dispersed data sources, and 3) fine temporal resolution. Here we describe the expansion of the BioFire® FilmArray® Syndromic Trends (Trend), a research epidemiology system containing exported data from BioFire® FilmArray® Respiratory Panel (RP) tests, from the United States to Colombia, South America. **Methods:** Data from over half a million FilmArray RP tests have been exported to the Trend database from 30 labs across the United States since 2013. In 2017, Trend was implemented and tested in four clinical laboratories across Colombia, allowing test results to be automatically exported from these clinical laboratories to the centralized Trend database. The pathogen detection and co-detection rates from these data were then contrasted to trends observed in data from clinical laboratories in the United States. **Results:** The BioFire® FilmArray® Systems participating in Colombia exported a total of 1,400 test results to the Trend database, dating back to November of 2015, with a majority of tests obtained from archived data. Overall RP positivity rate of the Colombian tests was 65% (95% confidence interval 61-69) compared to 50% (95% confidence interval 49-50) for the US. Tests with multiple detections were similar, with 10% of Colombian tests being positive for more than one pathogen, in contrast to 7% in the US. Individual pathogen detection rates were similar for the two regions, with the exception of Respiratory Syncytial Virus (RSV), which accounted for 25% of all positives in Colombia, contrasted with 7% of positive US samples. In 2016, the predominant Influenza A serotype in both Colombia and the US was H1-2009. In 2017, the predominant serotype was H3 for both locations. Type H3 is currently the predominating serotype in the US with Colombia yet to be determined. The respiratory season in Colombia appears to have two peaks roughly six months apart: one in late spring, the other in late fall. The late fall peak is primarily associated with RSV. For US sites, the respiratory season typically peaks in January or February, with RSV peaking in December. **Conclusion:** BioFire SyndromicTrends shows great promise in deciphering spatiotemporal dynamics of common respiratory pathogens. This epidemiological system can identify global differences in disease dynamics overtime. Future work with finer geographic distribution of contributing sites will aid in making conclusions regarding spatial dynamics of all 20 RP pathogens.

B-099

Detection of clinically relevant and unusual uropathogens obtained from urine culture from patients in Rio de Janeiro.

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Background: Urine culture is the most routine specimen in Clinical Microbiology laboratories. Urinary tract infections (UTI) are caused by a wide variety of uropathogens. Usually the clinical diagnosis of UTI is confirmed with the result of the urine culture associated with the result of urine sedimentation. Occasionally, disagreement between the results of both exams occurs, with altered sedimentation and negative culture. Pesence of fastidious microorganisms is one of the explanations for this disagreement because they do not grow in culture media routinely used in urine culture. The present study evaluated the detection of clinically relevant pathogens in urine culture from patients with sedimentation positive for nitrite and with pyuria and negative cultures. **Methods:** : From March to December 2017, 406,942 urine cultures were obtained from several units spread throughout the State of Rio de Janeiro, Brazil. Among these 37,919 (9.31%) were positive. However, in 114 patients, the urine had a positive nitrite and pyuria with no growth of microorganisms at culture. All urine cultures are routinely processed on CLED agar with a calibrated handle of 103 and incubated at 35 ° C for 48 hours. For those 114 negative urine cultures with pyuria and positive nitrite, we recultured the urine on a chocolate agar plate with a calibrated loop of 102 and incubated at 35 ° C in CO2 atmosphere for 48 hours to investigate nutritionally fastidious bacteria. **Results:** A total of 29 microorganisms were obtained from the agar chocolate plate of the 114 urine recultured. Only one bacteria was isolated in each of these 29 speci-

mens. They were identified by automated mass spectrometry (Vitek MS MALDI-TOF) method. 20 isolates were *Haemophilus* spp., 6 *Gardnerella vaginalis*, 2 *Oligella urethralis* and 1 *Streptococcus pneumoniae.* **Conclusion:**Many urine cultures without isolation of microorganisms, with altered sedimentation and with clinical diagnosis of urinary tract infection may present unusual uropathogens. It is important for all Laboratory of Microbiology to evaluate these cases, aiming at the recovery of these uropathogens. It is important that the microbiologist assess the result of sedimentation and the culture, looking for inconsistency between the both exams and in this case, routinely use another culture media in order to identify fastidious bacteria. A total of 29/114 (25,4%) urines with discrepancies between sedimentation and culture results would have a false negative culture if there was no active research of fastidious microorganisms implanted in the laboratory routine.

B-100

Anti-CMV IgM Antibodies on Filter Paper: An Alternative Approach to Internal Quality Control in Neonatal Screening.

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Background: The objective of the study was to verify if blood samples collected on filter paper can be routinely used as additional control because the commercial kit does not bring the presentation of controls in the same matrix of the neonatal samples. In addition to the liquid controls of the kit itself, extra controls were used in the 275 trials over a year, samples in DBS being 11 Reactive and 7 negative. **Methods:** Anti-cytomegalovirus IgM antibodies were checked in neonatal screening routine on dried blood samples collected on filter paper (DBS, S&S903) using ELISA-Serion Classic automated immunoassay. The Optical Density was measured at wavelengths 405 and 620 at 690 nm on Immunomat machine. **Results:** see the table bellow **Conclusion:** Reactive samples have compromised stability when subjected to successive cycles of refrigeration and exposure to room temperature. The measurement of DOs gradually declines day by day with the time of use, usually from day 7 and in a variable way, with reflection in the final calculation, translating into "gray area", justifying the high CV found (53,53). By observing the ODs of the assays, our consensus was that samples in DBS can and should be used as an alternative to internal quality control of the kit, being important parameter of the test because they will express on the same matrix the variations that are submitted to all samples.

Cytomegalovirus IgM - 275 dosages in DBS samples over 1 year in Neonatal Screening

	by comercial kyt			DBS samples (3,2mm punch)		
	Expected mean STDs (reference)	STD obtained	Negative liquid control (by kit)	Non-Reactive DBS (by Baby samples)	Reactive DBS (by Baby samples)	Reactive DBS (in house sample)*
O.D. min	0,450	0,625	0,005	0,004	0,286	1,515
O.D. max	1,615	1,547	0,127	0,148	2,179	2,210
O.D. medium	0,931	1,124	0,048	0,050	0,773	1,875
SD	0,04	0,11	0,01	0,03	0,32	0,16
CV	4,14	9,52	25,09	56,2	53,53	8,77

(*) In addition, a Reactive sample made in house was used in filter paper (equal parts IgM reactive of serum for cytomegalovirus with red blood cell concentrate). This sample had the highest reactivity index.

B-101

Testing anti-Zika virus NS1 IgA additionally to IgM increases sensitivity in acutely infected patients from regions endemic for flaviviruses

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Background: Specific IgM response to Zika virus (ZIKV) can be low or absent in patients with acute ZIKV infection and a history of other related flavivirus infections, e. g. with Dengue virus (DENV), presenting with an early high IgG titer. In these ZIKV cases, IgA against ZIKV non-structural protein 1 (NS1) was observed in the acute phase, suggesting anti-ZIKV IgA as alternative acute marker in secondary infections. In this study, we investigated the diagnostic benefit of an ELISA for combined detection of anti-ZIKV NS1 IgA and IgM. **Methods:** The following human serum panels were included in this study: 1) A sensitivity cohort (cohort 1) comprising acute serum samples (day 8-16 post symptom onset) of 31 residents from Colombia (2015), where ZIKV and DENV are endemic. Patients had been tested positive for ZIKV nucleic acid and anti-DENV IgG during the viraemic phase (≤ day 5). 2) A specificity cohort (cohort 2) consisting of serum samples (day 3-7 post symptom onset) of 40 Vietnamese patients, hospitalized with DENV hemorrhagic fever according to the World Health Organization case definition grade I and tested positive for DENV nucleic acid and anti-DENV IgG. Vietnam (2015) is endemic for DENV but not for ZIKV. Anti-ZIKV NS1 antibodies were determined in each sample using a commercial NS1-based Anti-Zika virus ELISA IgM (Euroimmun AG, Germany) and a corresponding ELISA (Euroimmun), applying a combination of anti-human IgA/IgM conjugated with peroxidase. **Results:** In cohort 1, 30 % (9/31) of samples were positive for anti-ZIKV NS1 IgM, whereas 100 % were positive for combined specific IgA and IgM. In cohort 2, none of the sera reacted in the Anti-Zika virus ELISA IgM, two samples were reactive in the Anti-Zika virus IgAM ELISA (5.0 %). **Conclusion:** Because patients with acute ZIKV infection from flavivirus endemic regions may not develop NS1-specific antibodies of class IgM, additional testing of anti-ZIKV NS1 IgA is required.

B-102

Multiplexed Host Response Biomarker Analysis on a Rapid, Quantitative Point-of-Care Platform

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Objective and Relevance. Literature suggests that host response biomarkers during acute infection may yield clinically relevant diagnostic or prognostic information. Rapid (< 30 min) detection of circulating protein biomarkers could provide actionable information during temporally complex conditions such as sepsis. Here we provide results for a rapid 3-plex host response marker assay run on a portable, point-of-care assay platform. The initial demonstration includes interleukin-6 (IL-6), procalcitonin (PCT), and C-reactive protein (CRP) in a single measurement. Data are presented for a collection of pediatric serum samples from patients clinically classified as sepsis, septic shock, and SIRS. **Methodology**. The MBio system consists of a disposable sample cartridge and portable reader for performing multiplexed fluorescence immunoassays. The cartridge-based assays combine a proprietary planar waveguide illumination approach with microarray-based spatial multiplexing and fluorescence imaging in a simple reader. The cartridge incorporates a fluidic channel with an array of capture antibodies. Workflow was as follows: each sample was mixed with a detection reagent comprising a cocktail of biotinylated antibodies and immediately added to the MBio cartridge. The mixture was incubated on-cartridge for 20 minutes, followed by a 10-minute streptavidin-fluorophore incubation. The IL-6 / PCT / CRP panel was selected to be representative of the range of host response markers that could be configured on the platform. Of note, we demonstrate simultaneous detection of a high concentration target (CRP > 30 micrograms/mL during inflammation) and a low concentration target (IL-6 limit of quantitation ~25 to pg/mL) in the same sample. **Clinical Sample Validation**. A collection of de-identified pediatric serum samples was provided by Dr. Hector Wong of the Cincinnati Children's Hospital. Samples were selected to include 10 from clinically identified pediatric sepsis patients, 10 SIRS, and 30 septic shock. Samples were run on the MBio platform, and reference ELISAs were performed for IL-6 and CRP. **Results**. Quantitative IL-6, PCT, and CRP results were generated on the MBio platform. There was overall correlation between MBio and the reference ELISAs. Three of 50 samples returned values beyond range (high) for the ELISA and MBio assays. Four samples were below detection limit for IL-6 on MBio. Most samples in the collection showed high PCT (> 0.5 ng/mL), as expected. 29 of 30 samples from septic shock patients showed PCT well above threshold. The one low PCT sample in this set showed significant hemolysis which may have affected the MBio result. The SIRS samples were also elevated in PCT, but were much less likely to be above threshold. These results suggest that the MBio assay is detecting differences in these clinically distinct categories. The CRP assay showed correlation with ELISA, but there were several sample with significant quantitative differences suggesting the CRP assay needs further optimization. CRP does not appear to be a discriminatory marker for the three clinical categories. **Conclusions**. Preliminary clinical sample data for this 3-plex assay suggest that the MBio platform can be used to deliver quantitative, protein biomarker panel results on clinically relevant samples in less than 30 minutes.

B-103

Detection of cytomegalovirus nucleic acid and mycoplasma nucleic acid in alveolar lavage fluid of pediatric patients with respiratory tract infection

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Background: There are limited reports on mixed infection of common atypical pathogens, so this study investigated the infection of human cytomegalovirus (HCMV) and mycoplasma pneumoniae (MP) in alveolar lavage fluid of pediatric patients with respiratory tract infection. **Methods:** A total of 31 pediatric patients with respiratory tract infection were enrolled in the Department of Pediatrics, the First Affiliated Hospital of Anhui Medical University from May to August in 2017, and the HCMV nucleic acid in the alveolar lavage fluid was detected by real-time fluorescent polymerase chain reaction. Ribonucleic acid (RNA) thermostatic amplification technology was used to detect MP nucleic acid in the alveolar lavage fluid. **Results:** The total detection rate of both pathogens was 64.52%, the positive rate of HCMV nucleic acid was 38.71%, and the positive rate of MP was 25.81%. **Conclusion:** HCMV and MP have high infection rate in pediatric patients with respiratory tract infection. The combined detection of these two infectious agents in alveolar lavage fluid has important application value for clinical etiology and treatment.

B-104

The mutable profile of infectious Candida species and resistance to antifungal agents: a clinical and laboratorial study

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Background: Vulvovaginitis by Candida spp, or vulvovaginal candidiasis CVV, is a common infection whose symptoms, located in the vulva and vagina, are characterized by intense pruritus and thick vaginal discharge, sometimes forming gums that adhere to the surface of the mucosa. This infection most often affects women of reproductive age throughout the world. However, the literature data on its incidence are incomplete because it is a non mandatory notification infection and because of the inaccuracy of the frequently used clinical diagnosis. To identify *Candida* species in patients with vulvovaginitis, determining their sensitivity to antifungal agents. **Methods:** were analyzed 84 vaginal secretion samples of patients seen at the Brasilia University Hospital Gynecology outpatient clinic. Nineteen patients were asymptomatic and 65 with vulvovaginitis, disclosing at least one of the following symptoms: vaginal discharge, vulvar hyperemia or edema, and localized itching or burning sensation. *Candida* phenotype was identified by culture, and confirmed by Matrix Assisted Laser Desorption Ionization Time-of-flight MALDI TOF. The sensitivity profile of *Candida* ssp for fluorocytosine, fluconazole, voriconazole, amphotericin B, capsofungin and mycofungin was determined by the Minimal Inhibitory Concentration MIC. **Results:** sample analysis of the 65 symptomatic patients showed 73% 48 positivity, with 75% 36 of the phenotypes identified as *Candida albicans*, 22.9% 11 as non-albicans species respectively, 8.3% of *C. glabrata*, 6.2% of *C. parapsilosis*, 4.2% of *C. tropicalis*, 2.1% of *C. krusei*, 2.1% of *C. Zeylanoides* and 2.1% of *Rhodotorula mucilaginosa*. In the antifungigram showed that *C. albicans* species were sensitive to all antifungal with the exception of one of the species that showed an intermediate sensitivity to amphotericin B 2.1%. Resistance was found among non-albicans species to fluconazole in 2.1% *C.glabrata*, to fluconazole in 2.1%, and to voriconazole in 2.1% *C. Krusei*. **Conclusion:** In view of significant increased infectivity of non-albicans species, with some phenotypes already showing resistance to usual antifungal agents, our results emphasize the need to precisely identify the *Candida* species, in order to abrogate possible treatment failure and repetitive episodes of vulvovaginitis.

B-105

Nucleic Acid Capture Using Silicon Dioxide Derivatized Magnetic Particles Provides the Foundation for Sensitive and Precise High Throughput Automated RT-PCR Assays

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Background: To address the need for nucleic acid capture in downstream and high throughput applications targeting the detection of viruses, we sought to develop magnetic particles (for research use only, not for use in diagnostic procedures) that would provide desirable analytical performance characteristics (limit of detection (LoD)), precision, and linear range) in automated RT-PCR assays and could be produced in large quantity in our own lab. **Methods:** Silicon-coated magnetic particles with a proprietary functionalization group were manufactured in our lab according to best practices. Approximately 1 milligram of magnetic particles are used per test to capture nucleic acids of lysed organisms. Each test was performed using K_2EDTA plasma or serum. Approximately 300 samples were collected and along with the particles, were loaded on to a fully automated processing instrument. Sample introduction, nucleic acid extraction, real-time PCR (RT-PCR) for HBV, HIV, HCV and CMV reaction setup, amplification and purification were performed without manual intervention. **Results:** The LOD results were 18 IU/mL for CMV in K_2ETDA plasma, 2.0 IU/mL for HBV K_2ETDA plasma and 3.8 IU/mL HBV in serum, 4.3 IU/mL for HCV and 30 IU/mL for HIV-1 both in K_2ETDA plasma. The standard deviation of the precision (Log IU/mL) was less than or equal to 0.16 for HBV, 0.15 for HCV, 0.16 for CMV and 0.20 Log cps/mL for HIV. The linear range (Log IU/mL) was 2.00-7.01 for CMV, 1.00-9.00 for HCV, 1.5-6.4 for HCV and 1.32-6.8 for HIV. **Conclusion:** The functionalized magnetic particles provide efficient DNA/RNA capture for high throughput detection and amplification of viral RNA using RT-PCR in a completely automated system. The extracted DNA/RNA from serum and plasma provides the basis for assays that have desirable performance characteristics that include: sensitivity, limit of detection, precision and linear range. We are currently evaluating the large scale production of the particles (0.6 Kg batches) and the use of the particles in other high throughput applications that require robust RNA/DNA extraction.

B-106

A Machine Learning Approach to Inflammatory Cytokine Profiling Reveals Diagnostic Signatures for Latent Tuberculosis Infection and Reactivation Risk Stratification

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Background: Latent tuberculosis infection (LTBI) is estimated in nearly one third of the world's population, and of those infected 10% will proceed to active tuberculosis (TB). Current diagnostics cannot definitively identify LTBI and provide no insight into reactivation risk, thereby defining an unmet diagnostic challenge of incredible global significance. However, by leveraging the unique immunological response to TB, a signature of cytokines may be useful for LTBI diagnostics. **Methods:** Using a silicon photonic microring resonator array, we developed and analytically characterized a 7-plex cytokine assay capable of automated screening of subject signatures in 46 minutes. This panel was used for profiling secreted immune response in LTBI-relevant samples from a 50-subject cohort with variable TB exposure risk. Peripheral blood mononuclear cells (PBMC) were isolated and immunologically challenged with five different stimulation conditions including two TB-specific antigens (CFP-10/ESAT-6, PPD) as well as three different controls representing positive (CD-3), negative (media), and off-target (*candida albicans*) immunological response. The panel of cytokine biomarkers was then quantitated in the supernatant of PBMCs from each stimulation condition. Additionally, all subjects were assessed for LTBI status and reactivation potential through standard-of-care diagnostic tests and regulatory guided classification. Absolute and control normalized responses for each biomarker (i.e. CD-3 stimulated response subtracted from CFP-10/ESAT-6 response) were evaluated for improved diagnostic capabilities using a machine learning guided feature selection algorithm. **Results:** Detection limits typically below 10 pg/ml, quantitation limits below 200 pg/ml, and inter-assay CVs at or below 10% were achieved with comparable response to ELISA. Boruta feature selection identified stimulated biomarker features that are predictive for LTBI and reactivation risk diagnoses. Normalized features, aiming to correct for differences in the basal immune state of each individual, were statistically revealed as unique from related stimulated responses and predictive for LTBI relative to healthy subjects. Notably, largely consistent signatures were identified for subjects with CDC defined LTBI as well as a stricter LTBI definition with IFN-ɣ, IP-10, IL-2, and CCL4 (under different combinations of stimulation normalization) showing strong predictive correlations. Orthogonal biomarker signatures were found to correlate with high and low reactivation risk. **Conclusions:** We developed and validated a multiplexed immunodiagnostic approach toward diagnosis of LTBI and stratification of reactivation risk that relies entirely upon secreted biomarker signatures from a simple in vitro assay. Multiplexed cytokine detection from within patient-derived samples of TB-related antigen exposure was performed using a silicon photonic platform that showed robust analytical performance. The biomarkers IFN-ɣ, IP-10, IL-2 appear as particularly promising markers for assessing LTBI status and TB reactivation risk when considered in light of comprehensive stimulation conditions and precision normalization for heterogeneities in basal immune response.

B-107

Comparison of clinical performance of SD Strep A Ultra Test and SD Strep A Rapid Test for diagnosis of acute bacterial pharyngitis

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Background: Rapid and accurate diagnosis of bacterial pharyngitis is essential for the optimal antibiotic treatment. Clinical performance of SD Strep A Ultra test (SD, Korea), a recently developed rapid antigen detection test (RADT), was evaluated for children with pharyngitis. **Methods:** Three-hundred forty three children with sore throat visiting seven pediatric clinics in Changwon, Korea were subjected to throat swabs twice during April-September, 2017. The first flocked swab was used for SD Strep A Ultra test. The other two cotton swabs were used for SD Strep A Rapid test and culture. PCR detecting *speB* gene was carried out for RADT-positive and culture-negative specimens. Clinical performance of SD Strep A Ultra was analyzed by the colony numbers and color intensity (range 1-20). The colony numbers were defined as 1+ for <10 CFU, 2+ for 10-50 CFU, 3+ for 51-100 CFU, 4+ for >100 CFU. This study was approved by IRB of Changwon Changwon Gyeongsang National University Hospital and all participants agreed on written consent. **Results:** Sensitivity, specificity, positive predictive value, and negative predictive value of SD Strep A Ultra were 97.4%, 90.8%, 93.0%, and 96.5%, respectively and those of SD Strep A Rapid were 95.8%, 94.7%, 95.8%, and 94.7%, respectively compared to throat culture. All three specimens showing RADT-positive and culture-negative were positive for the *speB* gene. When comparing with colony numbers, SD Strep A Ultra was negative with a frequency of 14.3% of 1+, 0% of 2+, 5.0% of 3+, and 0.9% of 4+ ($P = 0.021$). When comparing with the color intensity of SD Strep A Ultra, the frequency of GAS-negative was 11.5%, 15.9%, 3.9%, and 0% in the ranges of 1-5, 6-10, 11-15, and 16-20, respectively ($P < 0.001$). Area of ROC curve was 0.938 for the evaluation of diagnostic accuracy with color intensity of SD Strep A Ultra test. **Conclusions:** SD Strep A Ultra exhibited an excellent sensitivity and negative predictive value and comparable performance with SD Strep A Rapid. Discrepant result with culture might be due to different swab material (flocked swab and cotton swab), sampling order, bacterial numbers of GAS, and delayed transport.

B-108

Standardization of new indirect ELISA using a highly-specific egg protein from *Schistosoma mansoni* for diagnosis of different clinical forms in a low endemic area in Brazil

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Background: Schistosomiasis remains a global public health problem. In 2012, the WHO declared the elimination goal by 2020 and emphasized the need to develop highly accurate diagnostic tools adapted to low endemic areas. In Brazil the disease is caused by the species *Schistosoma mansoni* and is characterized by chronic low-intensity infections (<100 egg per gram of feces) in endemic areas and acute cases derived from internal migration and tourism. The "gold standard" method for WHO guidelines is the Kato-Katz, a stool microscopy-based technique which has low sensitivity in endemic areas of Brazil. In order to develop more sensitive tests, we searched for a specific marker and standardized by a conventional technique, enzyme-linked immunosorbent assay (ELISA). As a long-term goal, we intend to apply this marker on innovative technologies feasible to be used in low resource areas in a test-and-treat format. **Methods & Materials:** Using a protocol approved by the Brazilian Ethical Committee (n. 893.582), human serum was obtained from each group: healthy volunteers (negative controls); schistosome acute, chronic and post-treatment patients; and patients infected with other helminths. Fifteen samples from each group were pooled and submitted to two-dimensional Western blot (2D-WB) using native and sodium metaperiodate (SMP) treated schistosome soluble egg extract (SEA). The immunoreactive spots were identified by mass spectrometry and analyzed by bioinformatics tools. Recombinant protein of the selected biomarker was produced and applied to development of indirect ELISA using serum samples. **Results:** A total of 23 spots were identified. Among these, 22 spots were identified by serum from patients infected with other helminths, and 10 by negative control samples. Only 1 spot was recognized by *Schistosoma*-infected patients and detection remained after sugar denaturation by SMP. We identified this sequence (Major Egg Antigen), cloned using Gateway methodology, and produced the recombinant protein. The antibody detection by ELISA showed 88% sensitivity and 66% specificity in serum from Brazilian low-intensity infections. The next steps will be (1) ELISA evaluation using serum from different Brazilian endemic areas, (2) standardization using non-invasive samples to assess functionality, (3) production of monoclonal antibodies and evaluation of direct detection, and (4) development of a new point-of-care assay. **Conclusion:** The development of a new diagnostic requires long-term investments and we successfully achieved the initial steps. We identified a highly specific egg protein and showed its good performance in conventional ELISA making it promising candidate for improving Schistosomiasis diagnosis. We intend to develop innovative immunological methods using non-invasive samples as required by public sector. Furthermore, a test that is easier to use in the field will improve the accuracy for mapping of areas, to monitor impact strategies and perform post-elimination surveillance. We believe these new assays can potentially achieve the WHO guidelines and be included in elimination strategy programs used in affected countries.

B-109

Same-day checkup for active type of *Mycobacterium tuberculosis* complex by ultrasensitive ELISA

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Background: The definitive diagnosis has been believed to be performed with for tuberculosis. However, PCR detects not only active *Mycobacterium tuberculosis* (TB) complex but also nucleic acids obtained from dead TB. To fight tuberculosis, a rapid checkup for active type of TB complex is crucial. Recently, we have developed an ultrasensitive ELISA to detect proteins at 10^{-20} moles/test by use of enzyme cycling, in which a cycling reaction is conducted by a dehydrogenase (3α-hydroxysteroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androsterone derivatives). In the present study, we applied this ultrasensitive ELISA to a checkup for the active type of TB complex. Our proposed method provides the same-day (4-hour) results. Methods: We used MPB64, a specific protein secreted from active TB complex as a biomarker. BCG was used for the TB complex, and it was added into sputum obtained from people without tuberculosis. As a pre-treatment for the ultrasensitive ELISA, we warmed up BCG in the sputum and enhanced the secretion of MPB64. In the sandwich ELISA, two specific antibodies for MPB64 were used, one of which was conjugated with alkaline phosphate (ALP). An androsterone derivative with a phosphate was hydrolyzed by ALP, and this derivative was then employed in the enzyme cycling. Consequently, MPB64 could be determined by the accumulated amount of thio-NADH in the enzyme cycling. Results: The spike-and-recovery test using BCG and sputum demonstrated reasonable results. We succeeded in detecting TB (i.e., BCG) in the sputum at the level of 3×10^2 CFU/mL within only 4 hours. This rapidity can contribute to the prevention of disease spread, because potential patients can be isolated during the 4 hours that the results take. The present available tests for active TB detection are the sputum smear test and the sputum culture test. The smear test has low sensitivity (> 10,000 CFU/mL), whereas the culture test is highly sensitive (tens to hundreds CFU/mL) but requires a long culture period (at least 10 days). Furthermore, we applied our ultrasensitive ELISA to the sputum collected from the tuberculosis patients who had been already diagnosed with a BD BACTEC MGIT 960 Mycobacteria Culture System. The comparison results showed that the positive conformity ratio was 89% and that the negative conformity ratio was 98%. That is, the total conformity ratio was 95%. Conclusion: The present results showed that our ultrasensitive ELISA can be used enough to detect active TB complex within 4 hours. A conventional nucleic acid amplification test may detect dead bacteria, and it sometimes shows a false negative because of a small amount of bacteria in the sputum. Our present method, on the other hand, is a user-friendly ELISA without any specialized apparatus: it has almost the same sensitivity as the culture method but with same-day results. We believe that the detection of active TB complex within 4 hours enables us to judge the therapeutic effects.

B-110

Performance characteristics of the Alinity i HBsAg Qualitative II, Anti-HBc II and Anti-HBs assays utilized for routine laboratory Hepatitis B testing

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Background: Alinity i is a compact immunoassay system and a member of Abbott's next generation family of laboratory analyzers. Routine diagnosis of Hepatitis B Virus (HBV) infection is often assessed by using a panel consisting of HBsAg, Anti-HBc and Anti-HBs assays. The aim of the current study was to evaluate the key performance characteristics of these three assays that were developed for the Alinity i system. **Methods:** The Alinity i HBsAg Qualitative II, Anti-HBc II and Anti-HBs assays were tested side by side with the corresponding ARCHITECT assays. Analytical sensitivity for HBsAg, and Anti-HBc was determined using the corresponding WHO standards. A study to determine Limit of Blank (LoB) / Limit of Detection (LoD) / Limit of Quantitation (LoQ) for Anti-HBs was performed based on guidance from CLSI EP17-A2, whereas the measuring interval was determined based on guidance from CLSI EP06-A. Clinical specificity was assessed using unselected blood donor and routine diagnostic specimens, clinical sensitivity was determined using pedigreed positive specimens. **Results:** The analytical sensitivity of the Alinity i HBsAg Qualitative II assay was determined to be 19.93 - 20.87 mIU/mL (WHO 2nd IS, NIBSC code: 00/588). The Anti-HBc II assay exhibited an analytical sensitivity of 0.54 - 0.56 IU/mL on the WHO 1st IS (NIBSC code: 95/522). The clinical sensitivity of the Alinity i HBsAg Qualitative II assay was found to be 100,00 % using 496 known positive samples including different genotypes and mutants. The Alinity i Anti-HBc II assay also showed 100,00 % sensitivity, detecting all specimens from patients with acute, chronic and past/resolved HBV infection with anti-HBc antibodies. The specificity for blood donor specimens of the Alinity i assays under evaluation was 99.96% (5108/5110) for HBsAg Qualitative II and 99.86% (5162/5169) for Anti-HBc II. Similar values were found for the corresponding ARCHITECT assays (99.96% and 99.88%, respectively). Diagnostic specificity was found to be 100,00 % for HBsAg Qualitative II and Anti-HBc II on the Alinity i as well as on the ARCHITECT platform. The quantitative Alinity i Anti-HBs assay, standardized to the WHO 2nd International Reference Preparation, 2008 (code 07/164), had a LoB of 0.53 mIU/mL, LoD of 0.77 mIU/mL, and a LoQ of 2.00 mIU/mL. It showed performance within acceptance criteria for linearity, imprecision, and bias across the entire measuring range from 2.00 mIU/mL up to 1000.00 mIU/mL. Quantitative correlation between Alinity i and ARCHITECT Anti-HBs assays exhibited a slope of 1.08. **Conclusion:** The key performance characteristics of the three Alinity i assays used for routine Hepatitis B testing, HBsAg Qualitative II, Anti-HBc II and Anti-HBs are equivalent to the corresponding ARCHITECT assays. This will enable easy transition of existing ARCHITECT customers to the new Alinity i system that offers state of the art technology for increased operational efficiency.

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Quantitative Determination of Procalcitonin (PCT) In Human Serum by Lumipulse®G B•R•A•H•M•SPCT Assay

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INTRODUCTION: PCT (procalcitonin), a precursor of calcitonin, is synthesized by C-cells in the thyroid under normal conditions. Systemic inflammatory responses triggered by severe bacterial infections or sepsis, significantly increases synthesis of PCT resulting in elevated serum and plasma PCT levels. PCT is induced more strongly by bacterial infections compared to other inflammatory reactions (i.e. viral infections, autoimmune disease, transplant rejection, allergic reactions). Therefore, *in vitro* determination of PCT can be used to formulate differential diagnosis or to indicate severity of severe bacterial infections and sepsis. **METHODS:** The Lumipulse G B•R•A•H•M•S PCT is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of PCT in specimens on the LUMIPULSE G System by a two-step sandwich immunoassay method. PCT specifically binds to an anti-PCT monoclonal antibody (mouse) and anti-calcitonin monoclonal antibody (mouse) coated on particles and forms immunocomplexes. After washing, an alkaline phosphatase (ALP: calf)-labeled anti-katacalcin monoclonal antibody (mouse) specifically binds to PCT immunocomplexes, completing the sandwich. The amount of PCT is derived from the luminescence signals generated by adding the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt). Calibration of the Lumipulse G B•R•A•H•M•S PCT assay are traceable to in-house reference calibrators whose values have been assigned to Thermo-Fisher Scientific Inc.'s B•R•A•H•M•S PCT sensitive Kryptor. All verification and validation studies were performed according to respective CLSI guidelines. **RESULTS:** The Limit of Blank, Limit of Detection and Limit of Quantitation of the Lumipulse G B•R•A•H•M•S PCT assay was ≤0.0114 ng/ml. The Lumipulse G B•R•A•H•M•S PCT assay demonstrated linearity in the range from 0.010 to 104.260 ng/ml. There was no high-dose hook effect observed for samples containing up to ~12,000 ng/ml of PCT. A twenty-day precision study of 8 human serum-based panels and two commercially available serum-based controls assayed in duplicate at two separate times of the day using two LUMIPULSE G1200 systems (n = 80 for each sample) demonstrated within-laboratory (total) precision of ≤ 4.7%. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 9 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum albumin, immunoglobulin G, biotin, human anti-mouse antibody, and rheumatoid factor) and 26 commonly used therapeutic drugs. Cross- reactivity of the Lumipulse G B•R•A•H•M•S PCT assay with other substances (Human Calcitonin (10 ng/ml), Human Katacalcin (10 ng/ml), α-CGRP (10,000 ng/ml), β-CGRP (10,000 ng/ml), Salmon Calcitonin (13.2 µg/ml), and Eel Calcitonin (7.5 µg/ml), respectively) that are similar in structure to PCT demonstrated no cross-reactivity. A comparison of Lumipulse G B•R•A•H•M•S PCT with a FDA-cleared predicate device was analyzed using weighted Deming regression. For the 207 tested specimens (concentrations ranged from 0.054 to 58.156 ng/ml), the slope, y-intercept, and correlation coefficient (r) were 1.0199, -0.0044, and 0.9535, respectively. In a population of 213 self-reported healthy individuals, the 95th percentile, upper reference range limit was calculated at 0.045 ng/ml. **CONCLUSIONS:** The data demonstrate that the Lumipulse G B•R•A•H•M•S PCT assay on the automated LUMIPULSE G1200 System is sensitive, accurate and precise for routine quantitative determination of PCT in serum and plasma specimens.

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TLR1polymorphismsare significantly associatedwith the occurrence, presentation and drug-adverse reactions oftuberculosis in Western Chinese adults

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Background: Obtaining further knowledge regarding single nucleotide polymorphisms (SNPs) in the *TLR1* gene is of great importance to elucidate immunopathogenesis and management of tuberculosis (TB). **Methods:** We enrolled 646 tuberculosis patients and 475 healthy controls from West China. Six SNPs in *TLR1* were genotyped in every individual and were analyzed for their association with TB susceptibility and clinical presentation. The prospective follow-up was performed to determine whether these SNPs are associated with adverse reactions to anti-TB drugs. **Results:** Rs5743565 and rs5743557 were significantly associated with reduced predisposition to TB regarding the mutant allele in additive and dominant models with odds ratios (ORs) ranging from 0.61 to 0.83. There was increased tuberculosis risk associated with the haplotype CAG (rs4833095/rs76600635/rs5743596) [OR (95% CI) = 1.33 (1.07-1.65), p = 0.009] and with haplotype GG (rs56357984/rs5743557) [OR (95% CI) = 1.21 (1.02-1.43), p = 0.029]. The erythrocyte and hemoglobin levels were significantly higher in TB patients with the rs5743557 GG genotype than for AA and/or AG genotype carriers (p = 0.006 and 0.020, respectively). Chronic kidney damage and hepatotoxicity were common side-effects with RIF and INH regimens in this study with occurrence rates of 21.56% and 10.32%, respectively. Rs5743565 seemed to pose a higher risk of anti-TB-induced hepatotoxicity under the dominant model [OR (95% CI) = 2.17 (1.17-4.05), p = 0.013], and rs76600635 GG/AG genotypes were clearly correlated with the development of thrombocytopenia [OR (95% CI) = 2.98 (1.26-7.09), p = 0.010]. **Conclusions:** Rs5743565 and rs5743557 in the *TLR1* gene may contribute to decreased risk for tuberculosis susceptibility in a Western Chinese population. Rs5743565 and rs76600635 are potential risk factors for adverse reactions to anti-TB drugs. Our data help to characterize the development and progression of TB disease in China; however, multicenter studies and research into the precise mechanisms are needed to confirm these results. **Keywords:** tuberculosis; Toll-like receptor 1; single nucleotide polymorphisms; anti-TB drugs; adverse reactions; Western Chinese population

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Clinical Performance of the Bio-Rad BioPlex 2200 Toxoplasma gondii IgM assay

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Background: *Toxoplasma gondii* is a parasite that can be acquired following ingestion of cysts from feces of infected cats, or from eating undercooked or contaminated meat. Toxoplasmosis is routinely diagnosed through detection of *T. gondii*-specific antibodies. A major problem with *T. gondii*-specific IgM testing is lack of specificity, resulting in false positive IgM results. The BioPlex 2200 ToRC assays (IgM and IgG; Bio-Rad Laboratories, Hercules, CA) are multiplex flow immunoassays intended for identification of antibodies to *T. gondii*, Rubella and CMV in human serum or plasma. The BioPlex 2200 ToRC IgM assay is a new formulation that received FDA clearance in May 2017. Here we sought to evaluate the clinical performance of the *T. gondii* IgM portion of this assay. **Methods:** Two sample populations were utilized: 1) Prospective: 300 consecutive residual sera submitted for anti-*T. gondii* IgG and IgM testing as part of routine clinical care; 2) Archived: 52 residual sera previously positive for anti-*T. gondii* IgM and IgG using the predicate ADVIA Centaur *Toxoplasma* assays (Siemens, Malvern, PA). Performance of the BioPlex 2200 ToRC IgM and IgG assays was evaluated by calculating positive percent agreement (PPA) and negative percent agreement (NPA) compared to the Centaur tests. **Results:** Among the 300 prospective specimens the BioPlex 2200 assay demonstrated a percent negative agreement (NA) and positive agreement (PA) of 99.3% (288/290, 95% CI: 98.3-100%) and 0% (0/7), respectively, with the Centaur assay. Review of the medical record revealed that the 7 Centaur *T. gondii* IgM positive samples in this population were likely false positives. IgG demonstrated 95.8% (251/262, 95% CI: 93.4-98.2%) NA and 82.3% (28/34, 95% CI: 69.5-95.2%) PA in this population. Among the 52 archived samples positive for both IgG and IgM by the predicate method, the BioPlex 2200 IgM and IgG assays demonstrated a 90.4% (47/52; 95% CI, 82.3-98.4%) PA and 100% (52/52) PA, respectively. **Conclusions:** The BioPlex 2200 *T. gondii* IgM demonstrated excellent concordance with the ADVIA Centaur assay and may deliver fewer false positive results in a low prevalence population.

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Clinical performance of the Bio-Rad BioPlex 2200 Syphilis Total and RPR assay

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Introduction Syphilis infection caused by the spirochete, *Treponema pallidum*, is a major cause of sexually transmitted infections worldwide. Historically, serologic methods for the diagnosis of syphilis included a combined approach for the detection of antibodies to non-treponemal (RPR test) and treponemal (FTA-ABS or TP-PA test) antigens that are simple and reproducible but labor intensive. The BioPlex 2200 Syphilis Total and RPR assay (Bio-Rad Laboratories, Hercules, CA) was recently FDA-cleared and is a fully automated method for the simultaneous detection of treponemal and non-treponemal antibodies. Our objective was to evaluate the diagnostic performance of this assay at a tertiary medical center with a high rate of syphilis. **Methods** The study population consisted of 400 prospectively collected remnant serum specimens sent for syphilis testing as part of routine clinical care and 100 retrospectively collected RPR-positive specimens. Concordance of the BioPlex 2200 Syphilis Total & RPR assay to the predicate method was evaluated. The predicate method consisted of the Wampole RPR Card test with confirmation by the Inverness FTA-ABS test. Discrepant results were further tested using the Fujirebio Serodia TP-PA test. A titer was determined in any specimen positive by RPR. **Results** Of the 400 prospectively collected specimens, 263 (66%) were from females, of which 36 (14%) were pregnant, 166 (63%) were not pregnant, and 61 (23%) were of unknown pregnancy status. In total, 30 (8%) specimens were from HIV positive patients and the majority (81%) were 18 years of age or older. The positive and negative percent agreement (PPA and NPA) of the 400 prospectively collected specimens was 85% (17/20, 95% CI 84.5-85.5%) and 98% (373/380, 95% CI: 98.1-98.2%), respectively. The total concordance of the RPR results in the prospective population was 97.5% (390/400, 95% CI: 96-99%). Of the 3 potential false negative BioPlex RPR results, one specimen tested negative by both the confirmatory FTA-ABS predicate method and by TP-PA during discrepant analysis, suggesting that the negative BioPlex RPR result was true. Thus, the final result interpretation after confirmatory testing was 99% concordant (398/400) with the predicate method. The PPA of 100 predicate RPR positive retrospective samples was 88% (88/100, 95% CI: 87.8-88.2%). Of the 12 potentially false negative BioPlex RPR results, discrepant analysis by TP-PA revealed that 2 were likely falsely positive by the predicate FTA-ABS method. Seven of the 10 remaining potential false negative results were low positives with RPR titers < 1:4 by the predicate RPR method. For specimens with RPR titers determined using both the predicate and test method, there was a 79% agreement of the RPR titer within +/- one doubling dilution. **Conclusions**The performance of the BioPlex 2200 Syphilis Total and RPR assay was comparable to the predicate RPR and FTA-ABS methods. The high NPA of this assay, in combination with the ability to automate a historically labor intensive, make it well suited for use as a screen for syphilis in a high volume laboratory.

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Evaluation ofFilmarray for Early Diagnosis of Sepsis

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Background: Bloodstream infections (BSI) are one of the most important causes of death in healthcare settings. Accurate and rapid methods for the diagnostic of these infections are crucial for patient´s survival. Bacterial resistance is a major concern in these patients especially in nosocomial BSI **Methods:** We evaluated the platform Biofire Film Array (BioMérieux - Marcy l'Étoile - France) using the blood culture identification (BCID) panel for the diagnostic of BSI. This PCR based method is performed using positive blood culture bottles for identification of 19 bacteria, 5 species of *Candida* and also 3 resistance genes targets. We compared this method with conventional blood culture and Mass Spectrometry (MALDI TOF - MT) for identification of pathogens directly from positive bloodcultures. A total of 45 cases were selected. We carried out the tests at the same time and compared: the agreement of pathogens identification, detection of resistance genes and turnaround time (TAT) for the results **Results:** For identification, we found 84.5% and 78.0% of agreement when compared Biofire and MT with conventional culture, respectively. The Biofire missed one case (negative result) whose culture showed growth of *Roseomonas* sp. Partial agreement occurred in 3 cases: one the Biofire identified only *Enterobacteriacea* gender and the culture was positive for *Citrobacter* freundii; two cases there were growth of multiple agents and Biofire identified only one. Invalid results were observed in 3 cases (2 *E. coli* and one *Pseudomonas aeruginosa*). On the other hand, MT showed partial agreement in 2 cases where only one agent was identified and the cultured was positive for multiple agents. Invalid results were observed in 8 cases. For the resistance genes, Biofire identified a *mec*A gene in a *S. aureus* but in the culture oxacillin susceptible. Concerning about the time, Biofire and MT presented similar TAT for identification, significantly shorter compared to conventional culture. **Conclusion:** we conclude that Biofire presented an excellent performance for identification and detection of resistance genes. The limitation of Biofire is identification of agents no present in the panel. For resistance genes the Biofire provided a good correlation with the final susceptibility testing for the genes targeted.

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High Diversity of Yeasts Identified by MALDI TOF Mass Spectrometry in the Routine Clinical Microbiology Laboratory

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Background: Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has become a powerful tool for identification of pathogens, especially non-albicans Candida in clinical microbiology laboratories. The aim of this study was to evaluate the Vitek MS system (bioMérieux) as fast and reliable method for yeasts identification. **Methods:** From January to December 2017 we analyzed 18.854 fungi culture recovered from several clinical samples, including blood, peritoneal fluid, bronchoalveolar lavage, urine, wound and body fluid cultures. Clinical samples were cultivated first on Mycosel and Sabouraud-glucose agar, and then incubated at room temperature. All the isolates were identified by MALDI-TOF mass spectrometry using the Vitek-MS System which contains the MYLA database, according to the manufacture's recommendations. For calibration of equipment was used a reference strain of *Escherichia coli* ATCC 8739 according to the manufacturer's specifications. **Results:** A total of 1.310 (7%) yeasts were identified, including 434 (33.1%) *Candida albicans*, 490 (37.4%) non-albicans *Candida* and 386 (29.5%) yeasts identified at the species level. Overall, nine genera were identified. The predominant species of candida were: *C. albicans* (n=434), followed by *C. parapsilosis* (n=269), *C. tropicalis*

(n=44), *C. glabrata* (n=39), *C. guillermondii* (n=23), *C. famata* (n=22), *C. haemulonni* (n=21), *C. lusitaneae* (n=6), *C. norvegensis* (n=3), *C. catenulate*, *C. intermedia*, *C. krusei*, *C. lipolytica* and *C. pelliculosa* were identified in two samples, each and *C. dubliniensis*, *C. membranifaciens* and *C. pulcherrima* were identified in only one sample, each. 49 (3.74%) of the clinical isolates were identified only at the genus level as *Candida* spp. In the end, for other seven genus of yeast, the most prevalent were *Trichophyton* spp. (n=251), *Rhodotorula* spp., (n=53), *Tricosporon* spp., (n=30), *Cryptococcus* spp., (n=27) and *Microsporum* spp., (n=19) followed by *Geotrichum candidum* and *Hortaea werneckii* identified in one clinical sample, each. **Conclusion:** Our analysis demonstrated excellent rates for the identification of yeasts clinical isolates. Although, some limitations could be observed at the species identification level of *Candida* spp., the performance of MALDI-TOF MS technology is good for yeasts and the use of this methodology will provide direct benefits to the patient, through a more assertive empiric therapy, at a time when the rates of health care-associated infections have increased, especially by yeasts.

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TranscriptomeDifferences in Normal Human Bronchial Epithelial Cells in Response to Influenza A pdmH1N1 or H7N9 Virus Infection

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Background: In 2013, a novel reassortant influenza A virus (H7N9) of avian-origin emerged in the south of China has caused 800 human infections with a mortality of 40%. Although the first epidemic has subsided, the presence of a natural reservoir and the disease severity highlight the need to evaluate its risk on human public health and to understand the possible pathogenesis mechanism. Host factors might play a critical role in the development of severe complication. Normal human bronchial epithelial (NHBE) cell cultures had been proved to be an effective model to assess the viral host interaction. In this study, we aimed to assess host differential gene expression signatures in respiratory tract epithelial cells after influenza A virus pdmH1N1 or H7N9 infection. **Methods:** The NHBE cells cultured from a 24-year-old donor were challenged by 3.0 m.o.i. pdmH1N1, H7N9, or mock control. After 12 h and 36 h incubation, the cell pellets were collected for transcriptome analysis on the GeneChip HTA 2.0 array (Affymetrix platform); the bioinformatic softwares (MetaCore™, EC1.4, TAC 3.0) were used for results evaluation. All results were duplicated. **Results:** Results of principal components analysis showed that there were significant different transcriptome profiling patterns between pdmH1N1 and H7N9 at 12 h and 36 h post infection. Totally 44699 transcripts can be detected on HTA chip, compared with mock control, absolute fold change > 2.0 (FDR < 0.05) were evaluated. At 12 h post infection, 1937 (4.33%) transcripts in pdmH1N1 infected NHBE cells and 5325 (11.91%) transcripts in H7N9 infected cells significantly differentially expressed. At 36 h post infection, differential expression of transcripts in pdmH1N1 infected NHBE cells decreased (394 [0.88%]) whereas differentially expressed transcripts in H7N9 infected NHBE cells increased (6469 [14.47%]). Gene Ontology enrichment analysis revealed that the cellular repair related pathway which includes cytoskeleton remodeling pathway and keratin filaments pathway were significantly inhibited (keratin 4 gene expression fold change -640) in the H7N9 infected NHBE cells. However, the immune regulation related gene expression significantly increased in H7N9 infected group. **Conclusion:** Gene expression pattern in pdmH1N1-infected NHBE cells is significantly different from that in H7N9-infected NHBE cells. H7N9 virus infection induces stronger immune responses but damage cellular repair mechanisms at the same time. Our study results provide valuable insights to virus-host interactions between H7N9 and NHBE cells, which also help us having more understandings on the pathogenic mechanisms that lead to severe complications.

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Risk of HCV RNA Contamination by the cobas® e 602 Serology Module Prior to Nucleic Acid Testing by the cobas® HCV Test

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Background: Diagnosis of Hepatitis C involves antibody screening and confirmation of current infection by use of an HCV RNA nucleic acid test (NAT). Due to the potential risk for HCV RNA cross-contamination on serology devices employing a fixed needle for sample transfer, most laboratories either require a second blood draw from patients or a pre-serology aliquot from the primary serum specimen (in anticipation of a positive screen) in order to confirm active infection. As such constraints might jeopardize patient follow-up rates or place additional workflow burden on the lab, the ability to streamline the process and to allow the single specimen vial use for both testing procedures is of high importance. Here, we sought to assess the potential risk of HCV RNA cross-contamination by a serology screening instrument that employs disposable tips for sample transfer as an up-front process step to NAT confirmatory testing. **Methods:** Positive plasma specimens were generated by diluting armored HCV RNA (Roche Molecular Systems, Pleasanton, CA) at 6 Log and 7 Log IU/mL into normal human plasma (SeraCare Life Sciences, Millford, MA) to mimic high-positive clinical titers. Negative (n=60) and positive (n=60) plasma specimens were loaded onto the **cobas e** 602 module of the **cobas**® 8000 system in an alternating fashion and tested with the Elecsys® Anti-HCV II assay (Roche Diagnostics, Mannheim, DE); fresh negative specimens were loaded for an additional run, for a total of 120 negative cases. The HCV RNA-negative plasma samples were tested with the **cobas** HCV test for use with the **cobas**® 6800/8800 systems (Roche Molecular Systems, Branchburg, NJ) with a LoD of 8.5 IU/mL, to assess contamination potential of serology processing.**Results:** Testing of HCV RNA-negative plasma samples for the presence of low-level HCV RNA resulted in no detectable positive signal and an overall serology processing cross-contamination rate of 0% (95% Confidence Interval 0.00 - 0.03) (0/120). **Conclusion:** Hepatitis C antibody reactive specimens analyzed on the **cobas e** 602 serology module may be suitable for direct, primary specimen reflex testing by a sensitive HCV RNA confirmatory test, but additional studies are warranted. While this study design aimed to challenge the potential for contamination during serology processing by alternating high-positive and negative specimens, it does not mimic typical clinical laboratory presentation, which on average includes lower vial titers in a more randomized pattern. Nevertheless, the results herein demonstrate no risk of HCV RNA cross-contamination and that automated processes that minimize the need for manual intervention during the transfer of specimens, either prior to or after **cobas e** 602 assessment, may further reduce the chance of a contamination event.

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Monitoring the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children: protective anti-HBs levels and cellular immune responses

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Vaccination against hepatitis B virus (HBV) is recommended worldwide. The aim of this study was to assess the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children in the context of protective anti-HBs levels and cellular immune responses. Using a random questionnaire survey, 1,695 pre-school children were recruited as research subjects during January 2015 to June 2017. Blood samples were obtained to measure HBV serological markers as well as peripheral immunocytes. The children were divided into non-, low- and hyper- responsive groups (NR, LR, and HR) based on the vaccination efficacy. Additionally, the effect of revaccination on the NR group was evaluated at 1 month after completion of the vaccination course. Among a total of 1,695 children, 1,591 (93.86%) were infants who were followed while undergoing their primary course of hepatitis B vaccination at the 0-1-6 month schedule, and 1,249 (79.30%) of them developed antibodies against HBsAg (anti-HBs) titers greater than 10 IU/L. The results of immunocyte studies indicated that the $CD8^+$ T cells, $CD4^+CD45RO^+$ T cells, $CD8^+CD45RA^+$ T cells, and T follicular helper (Tfh) cells increased significantly in NR compared with HR. However, lymphocytes, $CD4^+$ T cells, and $CD4^+CD45RA^+$ T cells in NR were lower than that in HR. 96 of the non-response cases showed seroprotection after revaccination among 103 cases. Therefore, most of the preschool children who received hepatitis B vaccine in infancy achieved significant seroprotection. Seroconversion rates of individuals revaccinated after initial vaccination failure were significantly higher than those after primary vaccination. Different vaccination efficacy groups showed significant changes in circulating immunocytes, which might be a factor affecting the recombinant HBV vaccine's immune effectiveness.

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Diagnostic performance of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

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Background: Xpert MTB/RIF assay (Xpert) has the potential to accurately diagnose pulmonary tuberculosis in high and low burden countries. The purpose of this study was to evaluate the diagnostic performance of Xpert during routine clinical use in an intermediate burden setting. **Methods:** Between July 2014 and December 2016, a total of 2,952 consecutive respiratory specimens were simultaneously tested by Xpert, mycobacterial culture, and smear microscopy. **Results:** Compared with mycobacterial culture as the reference, the overall sensitivity, specificity, PPV, and NPV of Xpert were 74.1%, 97.5%, 74.7%, and 97.5%, whereas those of smear microscopy were 38.8%, 96.7%, 53.1%, and 94.2%, respectively. The sensitivity of Xpert was higher among smear-positive specimens compared with smear-negative specimens (96.1% [90.3-98.9] versus 60.2% [52.3-67.9], $P < 0.0001$), whereas the specificity of Xpert was lower among smear-positive specimens compared with smear-negative specimens (92.2% [84.6-96.8] versus 97.7% [97.1-98.3], $P < 0.01$). The sensitivity of smear microscopy was higher in early morning sputa compared with spot sputa (76.9% versus 35.2%, $P < 0.01$) and its specificity was higher in inpatients compared to outpatients (97.8% versus 94.7%, $P < 0.0001$). However, the diagnostic performance of Xpert was not affected by those factors of heterogeneity. **Conclusion:** Our data showed that performance of Xpert assay was more stable and superior to smear microscopy for diagnosis of pulmonary tuberculosis during routine clinical use in an intermediate tuberculosis burden setting.

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Quantitative capabilities of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

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Background: Xpert MTB/RIF assay (Xpert) has the potential to predict smear microscopy grade in high and low burden countries. The purpose of this study was to evaluate quantitative capabilities of Xpert for prediction of smear microscopy grade during routine clinical use in an intermediate burden setting. **Methods:** Between July 2014 and December 2016, a total of 2,952 consecutive respiratory specimens were simultaneously tested by Xpert, mycobacterial culture, and smear microscopy. **Results:** Among a total of 110 smear-positive specimens, 104 were Xpert-positive, corresponding to the overall sensitivity of 94.5%, whereas among a total of 2,576 smear-negative specimens, 2,422 were Xpert-negative, corresponding to the overall specificity of 94.0%. Among a total of 258 Xpert-positive specimens, 104 were smear-positive: Xpert semiquantitative results categorized as high, medium, low, and very low predicted 100% (15/15), 79.1% (53/67), 28.8% (30/104), and 8.3% (6/72) of smear-positive specimens, respectively, whereas Xpert predicted 99.8% (2,422/2,428) of smear-negative specimens. The semiquantitative result of Xpert had a strong correlation with smear microscopy grade for mycobacterial burden prediction (Goodman-Kruskal $\gamma = 0.982$, $P < 0.0001$). Among a total of 154 Xpert false-positive patients, 37 (24.0%) had cavitations on chest radiological findings, indicating high transmission potential of suspected pulmonary tuberculosis patients with Xpert false-positive results based on the initial negative smear examination. However, of 6 Xpert false-negative patients based on the initial positive smear examination, 1 (16.7%) presented pulmonary cavity, which suggested that Xpert-negative results could not perfectly rule out non-infectiousness of suspected pulmonary tuberculosis patients. **Conclusion:** Xpert semiquantitative results can provide a novel standardized strategy to measuring bacillary load in the sputum of patients with pulmonary tuberculosis.

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Detection and quantification of Hepatitis C Virus using the new Aptima HCV Quant Dx assay in the fully automated Panther® System compared to the Abbott Realtime HCV assay.

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Background: Hepatitis C Virus (HCV) continues to be an important health concern worldwide. Different therapeutic methods are now available for the treatment of HCV infection with good results. Determining the viral load of patients under treatment is now the standard of care for monitoring the response to these treatments. There are different commercially available assays used to detect and quantify HCV RNA in serum and plasma specimens. The objective of this study was to compare the Aptima HCV Quant Dx assay, recently released by Hologic, Inc®, with the Abbott Molecular Realtime HCV assay. The Aptima HCV Quant Dx assay is a real-time transcription-mediated amplification (TMA) test, run in the Panther System (Hologic) used for confirmation of diagnosis and monitoring of HCV RNA. The Abbott Realtime HCV assay is an RT-PCR test run on the automated m2000 system (Abbott Diagnostics). **Methods**: Sixty plasma specimens, twenty negatives and forty positives for HCV were included in this study. All sixty specimens were used to test the qualitative performance and thirty of them, with known viral loads, were used to test the quantitative performance. All these specimens had been previously tested on the Abbott m2000 platform. The specimens were assayed using the Aptima HCV Quant Dx Assay on the Panther System following the manufacturer instructions. Specificity of the new assay was tested using 20 HCV negative specimens, some of which were positive for Cytomegalovirus (CMV) and Human Immunodeficiency Virus (HIV). Precision was tested using a known HCV positive specimen repeated twelve times in different runs. Results obtained from specimens tested in both instruments were compared using the EP Evaluator program. **Results**: The EP Evaluator software was used to determine whether the methods are equivalent within a total allowable error of 1 $\log_{10}$ IU/mL. Thirty specimens with known HCV genotypes 1a, 1b, 2b, and 3a were compared over a range of 1.11 to 6.98 $\log_{10}$ IU/mL. The test passed with 98.3 % agreement. One specimen with low viral load was negative on the Abbott instrument and positive on the Panther system. This could be explained because the Aptima HCV Quant Dx Assay has a lower detection limit (<3.9 IU/mL) than the Abbott System (<12 IU/mL) in plasma specimens. The difference between the two methods was within allowable error. The average error index was 0.12 with a range of -0.41 and 0.54. The coefficient of correlation (R) between both methods was 0.9951. For the precision study, the EP Evaluator results showed a mean of 3.861 $\log_{10}$ IU/mL with a standard deviation of 0.047. This value was within the 2 SD range (3.767-3.954). **Conclusions:** We can conclude that the Aptima HCV Quant Dx assay is a highly sensitive, accurate, and reproducible assay with a performance equal to that of the Abbott Realtime HCV assay. The Aptima HCV Quant Dx assay is a faster and more efficient test than the latter. This is helpful in the lab setting because it reduces hands on time needed to set up the test and allows for shorter wait time for results.

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Method comparison of the VITROS ® Immunodiagnostic Products Anti-*T. cruzi* (Chagas) Assay* to the Ortho® *T. cruzi* ELISA Test System

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Background: This study was designed to compare the clinical performance of the VITROS Immunodiagnostic Products Anti-*T. cruzi* (Chagas) assay (VITROS Anti-*T. cruzi* assay)* to the FDA licensed and CE-marked Ortho *T. cruzi* ELISA Test System (Ortho *T. cruzi* ELISA). **Methods:** All testing in this study on the VITROS Anti-*T. cruzi* assay* was split across the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated Systems. The Ortho *T. cruzi* ELISA testing was performed on the Verseia Integrated Processor. The clinical samples included 5210 human serum and plasma samples, including 200 presumed negative hospitalized patient samples and 5010 low risk blood donor samples, 418 presumed *T. cruzi* serological positive samples and 63 samples from subjects characterized as parasite positive by historical identification of *T. cruzi* parasites. The presumed serological positive samples were determined to be reactive on at least two other serological methods prior to this study. **Results:** For the 5010 low risk blood donor samples there was 100% agreement between methods with all samples being non-reactive with both methods. For the 200 hospital-

ized patient samples there was also 100% agreement between methods with all samples being non-reactive with both methods. For the 63 parasite positive samples there was 100% agreement between methods with all samples being reactive on both methods. For the 418 presumed serological positive samples there was 100% agreement between methods with all 418 samples being reactive on both methods. This resulted in an overall agreement of 100% for these 5691 reactive and non-reactive clinical samples. **Conclusion:** The VITROS Anti-*T. cruzi* (Chagas) assay* demonstrated equivalent clinical performance in the detection of *T. cruzi* antibodies to the FDA licensed and CE-marked Ortho *T. cruzi* ELISA Test System.*Under development.

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An Evaluation of Performance of the VITROS ® Immunodiagnostic Products Anti-*T. cruzi* (Chagas) Assay*

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Background: This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products Anti-*T. cruzi* (Chagas) assay (VITROS Anti-*T. cruzi* assay)* on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated Systems. The assay detects human antibodies to *Trypanosoma cruzi*, the causative agent of Chagas' disease. **Methods:** Antibody detection in the VITROS Anti-*T. cruzi* assay* is achieved using lysate antigens coated onto the well. Sample is added to the coated wells in the first stage of the reaction, and *T. cruzi* antibody from the sample is captured. After washing, HRP conjugated murine monoclonal anti-human IgG antibodies are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. Specificity was assessed using 5210 human serum and plasma samples, including 200 presumed negative hospitalized patient samples and 5010 blood donor samples. Sensitivity was evaluated using 63 samples from subjects characterized as parasite positive by historical identification of *T. cruzi* parasites. Seroconversion sensitivity was assessed by testing a commercially available panel. Assay reproducibility was assessed using two reagent lots with a 5 member panel. Analytical sensitivity was determined by testing serial dilutions of the WHO 1st International Standard (*T. cruzi* I and II) for Chagas in three determinations across two reagent lots. **Results:** The specificity of the VITROS Anti-*T. cruzi* assay* for the combined blood donor and hospitalized patient populations was 100.0% (5210/5210) [95% exact CI (99.93-100.00%)]. The sensitivity for parasite positive samples was 100.0% (63/63) [exact 95% CI (99.3-100.0%)]. For the seroconversion panel all seropositive bleeds were reactive. For the reproducibility study the observed precision for the 4 reactive panel members ranged from 2.8 to 9.0 %CV. The overall sensitivity for the WHO Chagas (anti-*Trypanosoma cruzi* I) antibody standard (09/188) was a mean of 31.2 mIU/mL (range 27.2 to 35.5 mIU/mL) with a calculated endpoint titer mean of 32.4 (range 28.2 to 36.8). The overall sensitivity for the WHO Chagas (anti-*Trypanosoma cruzi* II) antibody standard (09/186) was a mean of 59.5 mIU/mL (range 54.4 to 63.6 mIU/mL) with a calculated endpoint titer mean of 32.4 (range 28.2 to 36.8). **Conclusion:** The VITROS Anti-*T. cruzi* Assay* demonstrates excellent clinical and analytical performance in the detection of human *T. cruzi* antibodies. *Under development

B-125

Novel ELISA based on antigens from Strongyloides papillosus instead of Strongyloides ratti exhibits increased serological specificity

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Background: Strongyloidiasis is an infectious disease caused by the nematode Strongyloides. Human infection by Strongyloides stercoralis can manifest with dermatological, intestinal and pulmonal symptoms frequently passing into a chronic disease. Low parasitic loads and discontinuous larvae excretion may hamper diagnosis by coproscopy. Serological test systems are more sensitive to detect the infection. Available serological tests are commonly based on native antigens from S. ratti larvae and lack specificity. We developed and evaluated the first ELISA based on S. papillosus to increase specificity. **Methods:** Evaluation of the ELISA based on S. papillosus was performed using the following three approaches: [1] Participation in an external quality assessment scheme (NEQAS, UK) encompassing six positive and five negative samples [2] A correlation study with the commercial Bordier ELISA (Strongyloides ELISA kit based on S. ratti antigens; Bordier Affinity Products, Switzerland) including 89 sera pre-characterized as either positive (n = 59) or negative (n = 30) by means of Bordier ELISA [3] Comparison with an in house ELISA based on S. ratti by determining specificity with respect to a cross-reactivity panel (n = 193, samples from patients with other parasitic or bacterial infections) and a control panel (n = 688, samples from 500 healthy blood donors, 100 pregnant women and 88 children) **Results:** [1] Results obtained with the Anti-Strongyloides ELISA were 100 % in agreement with NEQAS target values. [2] In 74 of 89 samples (83,1%), the result of the novel ELISA correlated with the Bordier ELISA. Seven discrepant cases, which were positive in Bordier ELISA but negative in the novel ELISA, were further examined. Serological analyses indicated the presence of antibodies against other parasites (Plasmodium spp., Schistosoma spp. and Echinococcus spp.) in six of these cases. [3] The S. ratti based ELISA was reactive in 13,9% of the sera in the cross-reactivity panel and in 10,6% of the samples from healthy individuals, yielding a combined specificity of 88,6 %. In comparison, reactivities of 6,2% (cross-reactivity panel) and 3,5% (healthy individuals) were detected with the novel Anti-Strongyloides ELISA, resulting in a combined specificity of 95,9%. **Conclusion:** The novel Anti-Strongyloides ELISA reveals a high diagnostic accuracy in the serological diagnosis of Strongyloidiasis. The use of native antigens from S. papillosus instead of S. ratti increases assay specificity by 7,3%.

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Recombinant antigens improve sensitivity and allow species differentiation in echinococcosis diagnostics

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Background: Cystic and alveolar echinococcosis (CE and AE) are caused by the tapeworms Echinococcus granulosus and Echinococcus multilocularis, respectively. Serological tests should be used before invasive methods according to CDC guidelines. For species differentiation, blot techniques using specific recombinant antigens are of increasing significance. **Methods:** We tested 329 clinically and serologically (ELISA or Western blot) pre-characterized sera. Among these were 55 CE and 52 AE samples, 122 samples of patients with other parasitic infections (including the following species: Fasciola hepatica, Strongyloides stercoralis, Taenia solium, Trichinella spiralis, Schistosoma ssp., Plasmodium ssp., Toxocara spp., Entamoeba histolytica, Leishmania ssp., Ascaris lumbricoides, Anisakis simplex and Filarioidea types), 50 healthy blood donors and 50 tumor patients. Anti-Echinococcus species-specific IgG was determined using a Western blot with electrophoretically separated Echinococcus multilocularis metacestode vesicle fluid (EmVF) and 3 membrane chips coated with recombinant E. granulosus antigen EgAgB and E. multilocularis antigens Em18 and Em95. Bands were automatically evaluated using a commercial software (EUROLineScan, Euroimmun). **Results:** Testing the pre-characterized patient sera, the conventional Western blot achieved a sensitivity of 89% at a specificity of 100% for echinococcosis, and with added recombinant proteins an increased sensitivity of 93% (at 100% specificity). Since the evaluation is challenging, a specific algorithm for species differentiation was designed in the EUROLineScan software on the basis of the antibody findings. In patients positive for specific anti-Echinococcus ssp. antibodies, the causative species was correctly assigned by the software as E. multilocularis or E. granulosus in 33 of 45 AE patients and 45 of 52 CE patients, respectively. The recombinant antigens showed no cross-reactivity, while, in the Western blot, positive results were obtained in 6 out of 122 samples and only in Anisakis and Ascaris cases. **Conclusion:** Supplementing the EmVF Western blot with immobilized recombinant antigens (Anti-Echinococcus EUROLINE-WB) increases the sensitivity for echinococcosis, at a constantly high specificity, and enables differentiation between Echinococcus species. Furthermore, no cross-reactivity to diagnostically highly relevant Taenia solium, Schistosoma ssp. and Entamoeba histolytica was observed.

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Improved Sensitivity for Detection of Urinary Tract Infections Using Novel Light Scattering Methodology

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Background: Urinary tract infection (UTI) is common, and urine culture is one of the highest volume tests performed in clinical microbiology laboratories. Overuse of culture can not only monopolize laboratory resources, but also lead to unnecessary antimicrobial exposure as patients may receive treatment while await-

ing culture results, putting them at risk for *Clostridium difficile* infection and adverse side effects of medications as well as promoting bacterial antimicrobial resistance. A common approach to decrease unnecessary urine culture is to screen samples using urinalysis (UA) parameters to determine those that should proceed to culture (reflex), though guidelines to optimize sensitivity for UTI detection and specificity to eliminate unnecessary culture have not been defined. The objective of this study is to compare a novel UTI detection method (BacterioScan 216Dx UTI System) to urinalysis for screening urine samples for reflex to culture. **Methods:** Urine samples (n=124) submitted for culture were evaluated by urinalysis and a novel laser light scattering device (216Dx) used to detect the presence/absence of UTI pathogens in urine after dilution in Tryptic Soy Broth (TSB) and 190 minutes of optical assessment. Reflex parameters for culture as defined in our institution were compared to results from 216Dx to evaluate sensitivity and specificity for UTI detection defined as growth in culture of one or two uropathogens at concentrations of ≥10,000 CFU/ml. **Results:** 124 urine samples were evaluated by UA, culture and 216Dx. The 216Dx demonstrated a 100% sensitivity and 82.24% specificity for the detection of UTI, compared to UA at a sensitivity of 88.24% and a specificity of 71.96%. **Conclusion:** Screening tests are optimized for sensitivity to decrease the risk of false negative results that may harm patients. However, as specificity decreases more samples must proceed to confirmatory testing. In this study, UA demonstrated a sub-optimal sensitivity and specificity with 2 false negatives and 30 false positives. The use of a novel screening method to evaluate whether urine samples should proceed to culture provided optimal sensitivity (100%) and an improved specificity leading to 0 false negatives and fewer false positives (19 vs 30). This screening approach could lead to improved antimicrobial stewardship and patient care through fewer patients receiving antimicrobial agents while awaiting urine culture results.

B-128

Genetic polymorphisms of long non-coding RNA *RP11-37B2.1* associate with susceptibility of tuberculosis and adverse events of anti-tuberculosis drugs in the Western Chinese Han population

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Background: Little knowledge about the biological functions of *RP11-37B2.1*, a newly defined long non-coding RNA (lncRNA) molecule, is currently available. Previous studies have showed that rs160441, located in the *RP11-37B2.1* gene, is significantly associated with tuberculosis (TB) both in a Ghanaian and the Gambian populations. **Methods:** We investigated the influence of several SNPs within lncRNA *RP11-37B2.1* on the risk and manifestations of TB and the possible correlation with adverse drug reactions (ADRs) from TB treatment in a Western Chinese population. Five SNPs within lncRNA *RP11-37B2.1* were genotyped in 554 TB patients and 561 healthy subjects using the improved multiplex ligation detection reaction (iMLDR) method, and the patients were followed up monthly to monitor the development of ADRs. **Results:** No significant association between the SNPs of lncRNA *RP11-37B2.1* and TB susceptibility was observed in total samples (all p values > 0.05). Surprisingly, significant associations were observed between rs160441, rs218916 and rs218936 and thrombocytopenia development during anti-TB therapy under the dominant model (rs160441: CC: 2.42% vs. CT + TT: 7.32%; rs218916: CC: 1.46% vs. CT + TT: 7.29%; rs218936: CC: 2.25% vs. CT + TT: 6.92%, respectively) with the estimated $p = 0.014$ [odds ratio (OR) = 3.18], 0.003 (OR = 5.32) and 0.018 (OR = 3.23), respectively. **Conclusion:** Our findings firstly exhibit that three lncRNA *RP11-37B2.1* SNPs significantly associate with the occurrence of thrombocytopenia and suggest *RP11-37B2.1* genetic variants may potentially act as the useful biosignatures for identifying TB patients at greater risk of thrombocytopenia development during anti-TB treatment.

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Evaluation of several FDA-cleared *Borrelia burgdorferi* ELISAs within modified two-tiered testing algorithms

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Background: Serology testing for *Borrelia burgdorferi* infection consists of a two-stage algorithm, referred to as standard two-tiered testing (STTT). In the STTT algorithm, specimens are initially assayed by methodologies such as IFA or ELISA, and equivocal or positive specimens are subsequently tested via immunoblotting for detection of IgG and/or IgM antibodies. While specific, the immunoblotting portion of the STTT algorithm contains drawbacks such as insensitivity for detecting acute infection, subjectivity of result interpretation, and technically challenging procedures. Consequently, there are published studies that support the replacement of immunoblotting with a more sensitive and automatable methodology such as ELISA; referred to as modified two-tiered testing (MTTT). The objective of this study was to evaluate the performance of several FDA-cleared *Borrelia burgdorferi* ELISAs within MTTT algorithms. **Methods:** 280 clinically-characterized and blind-coded serum samples (termed Premarketing Panel), were obtained from the Centers for Disease Control and Prevention (CDC). The Premarketing Panel was initially tested by the *Borrelia burgdorferi* IgG/IgM ELISA Test System (ZEUS Scientific, part# 3Z9651), as well as the VlsE1/pepC10 IgG/IgM ELISA Test System (ZEUS Scientific, part# 3Z9661), and the raw data were submitted to the CDC for decoding and assembly relative to preexisting clinical and STTT data (VIDAS®/Marblot™). The Premarketing Panel was subsequently tested by the *Borrelia burgdorferi* IgG ELISA Test System (ZEUS Scientific, part# 3Z9651G), *Borrelia burgdorferi* IgM ELISA Test System (ZEUS Scientific, part# 3Z9651M), and the C6 Lyme ELISA™ (Immunetics, cat# DK-E352-096). The ELISA data derived from testing the Premarketing Panel were assembled and analyzed according to the following six MTTT algorithms: [1. 1st Tier - *Borrelia burgdorferi* IgG/IgM ELISA, 2nd Tier - VlsE1/pepC10 IgG/IgM ELISA], [2. 1st Tier - *Borrelia burgdorferi* IgG/IgM ELISA, 2nd Tier - C6 ELISA], [3. 1st Tier - *Borrelia burgdorferi* IgG and/or IgM ELISA composite result, 2nd Tier - VlsE1/pepC10 IgG/IgM ELISA], [4. 1st Tier - *Borrelia burgdorferi* IgG and/or IgM ELISA composite result, 2nd Tier - C6 ELISA], [5. 1st Tier – VIDAS IgG/IgM, 2nd Tier - VlsE1/pepC10 IgG/IgM ELISA], [6. 1st Tier - VIDAS IgG/IgM, 2nd Tier - C6 ELISA]. **Results:** Of the 30 acute Lyme disease samples, the STTT algorithm detected 14 (47%), and the MTTT algorithms (numbered 1-6 above) detected 22 (73%), 20 (67%), 23 (77%), 21 (70%), 21 (70%), and 20 (67%) respectively. Of the 90 total Lyme disease samples (representing disease stages 1-3), the STTT algorithm detected 66 (73%), whereas the MTTT algorithms (numbered 1-6 above) detected 77 (86%), 75 (83%), 80 (89%), 78 (87%), 76 (84%), and 75 (83%) respectively. Of the 190 non-Lyme disease control samples, the STTT algorithm detected 0 (0%), and the MTTT algorithms (numbered 1-6 above) detected 2 (1.1%), 0 (0%), 2 (1.1%), 1 (0.5%), 3 (1.6%), and 1 (0.5%) respectively. **Conclusion:** This study represents the first MTTT evaluation of several ELISAs that are currently FDA-cleared for use as 1st tier tests. The novel data presented herein are consistent with previously published literature, and support the notion that the MTTT algorithm yields improved sensitivity for detection of early Lyme disease, while maintaining acceptable specificity.

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Universal Pathogen Capture System for Rapid Isolation of Intact Bacteria Directly from a Patient Sample

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Background: Currently, patient blood samples have to be cultured prior to performing genetic, mass spectrometric, or phenotypic analyses for bacterial identification. While recent methods have reduced the time to identify the bacteria, the rate-limiting step is still blood culturing. Culture-independent methods rely on antibody or DNA-based recognition for capturing the bacteria. While in principle a large number of bacteria can be captured, in practice, the number of pathogens captured are limited to those that can be recognized by the antibody/ DNA on the chip/kit, which is a small number. Further, these methods are unable to process the entire patient sample volume which lowers their overall diagnostic accuracy. We report a new approach to isolate intact bacteria directly from patient blood samples in < 1 hour, which does not rely on antibodies or DNA primers. The system isolates intact bacteria by selectively breaking down blood cells but not bacteria, separating the intact bacteria from the lysis debris by filtration, and concentrating them for multiplex analysis. The very highly selective lysis of blood cells is accomplished by leveraging the differential response of blood cells and bacteria to mechanical forces while the filtration is performed using ultrathin defined-pore membranes. **Objective**: To evaluate the performance of this approach for isolating bacteria directly from blood. **Methods**: Bacterial strains were obtained from collaborators and ATCC and cultured prior to spiking different concentrations into 5-10 mL of whole blood containing disodium EDTA. The seeded samples were passaged through the selective lysis unit of the platform. The lysate was filtered to eliminate the lysis debris and to concentrate the bacteria. Lysis efficiency was monitored by microscopy as well as by dynamic light scattering. Bacterial recovery was measured by enumerating bacteria in the seeded sample as well as in the lysate, filtrate, and filter surface. **Results**: A total of 25 bacterial strains were analyzed that included gram positive species such as *S. aureus* and *E. faecium* and gram-negative species such as *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa*. The isolation of intact bacteria from 10mL of whole blood was completed in < 50 minutes. Recovery of intact bacteria after passage through the lysis unit was > 90% and through the full process was > 85%. Bacteria were viable after isolation. **Conclusion**: The approach offers the ability to isolate bacteria directly from whole blood in < 50 minutes without utilizing

antibodies or DNA primers. This, in turn, has the potential to both isolate a broad range of bacteria as well as to dramatically reduce the time for microbial identification by eliminating the need to culture the blood sample. The isolated bacteria can be identified by any suitable method. Further studies are needed to directly identify bacteria from clinical samples and to expand the number of pathogens analyzed. These investigations will aid in the development of a universal pathogen capture system that can help isolate and identify a broad range of bacteria directly from blood in < 1 hour.

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Effects of erythropoietin on experimental Chagas disease: histopathological and cardiac biomarkers.

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Background: Chagas disease caused by the protozoan *Trypanosoma cruzi* represents a serious health public problem with high morbidity and consequent elevated mortality rate. About seven million people are infected with the parasite, mostly in Latin America. Approximately 30% of individual with chronic infection develop Chagas cardiomyopathy, the most important clinical manifestation of the disease. Chagasic cardiomyopathy presents different forms, but all of them culminate with cardiac dysfunction due to cardiomegaly, myocarditis and cardiac fibrosis. Trypanocidal drugs, despite reducing parasitaemia, have no efficacy on the progression of lesions in the chagasic heart. In this way, there is no effective treatment for these cases, condemning the patient to live with or succumb to the disease. Erythropoietin (Epo), a key-regulator of erythropoiesis, also has a cardioprotective effect by reducing the processes of apoptosis, inflammation and myocardial ischemia through the formation of new blood vessels. However, it is unknown whether the action of this protein can be effectively used both in prevention or treatment of Chagas cardiomyopathy. Thereby, this study aims to assess the possible cardioprotective effect of erythropoietin (Epo) on experimental chronic Chagas disease. **Methods:** C57BL/6 mice were randomly divided into four groups: administration of saline or Epo during 30 days before the infection (to verify the protective effect of Epo) and administration of saline or Epo during the acute phase of the disease (30dpi) (to verify the therapeutic effect of Epo). All the animals were infected by intraperitoneal route with 10^5 trypomastigotes Colombiana strain of *T. cruzi*. The activity of biomarkers of heart lesion (total creatine kinase – CK, myocardial fraction of CK – CKMB and aspartate aminotransferase – AST) were measured in blood samples before the infection (D0) and 15, 30, 90 and 180 dpi. Histopathological analysis (haematoxylin and eosin) of heart, spleen and large intestine on the chronic phase of Chagas disease (180 dpi) were performed. **Results:** A therapeutic effect of Epo was observed in CK total ($p<0.001$), but this did not occur in the cardiac muscle fraction of CK. In fact, the kinetics for CKMB activity throughout the *T. cruzi* infection determined in mice treated or no with Epo showed no differences. Concerning the AST activity, infected mice treated with Epo in the acute phase had increased levels in the course of the disease when compared to the D0 ($p<0.001$). The histopathological analysis showed lesions in evaluated tissues (heart, spleen and intestine) in all experimental groups. However, no significant difference was seemed between groups. **Conclusion:** Thus, the administration of Epo during 30 days before the *T. cruzi* infection or during the first 30 dpi does not prevent the occurrence of cardiac damage in Chagas disease.

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Automated and laboratory information system integrated workflow for detection of yellow fever virus by RT-qPCR in EDTA-plasma.

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Background: In December 2016, Brazil reported the country's largest yellow fever virus (YFV) outbreak in decades. Through 16 February 2018, about 5000 suspected cases were reported, including 500 deaths. Immunologic detection methods for YFV, such as MAC-ELISA, can cross-react with others Flavivirus. Conversely, RT-qPCR can reliably and accurately detect YFV during the viremic phase (5-7 days after the symptoms-onset). RT-qPCR main drawback is its production capacity so that the development automated of tests is crucial. The present study aimed to validate an automated RT-qPCR assay for YFV detection. **Methods:** Specimens used in this validation were quality control materials positive for YFV by MAC-ELISA (Serum, n=15) donated by a Brazilian external quality assurance provider (Controllab) and EDTA-plasma samples from healthy volunteers (n=96). The control materials were tested for YFV by RT-qPCR and pooled. The pool's viral load was quantified against serial dilution of synthetic ssDNA corresponding to the YFV RT-qPCR genomic target. EDTA-plasma samples from the healthy volunteers were spiked with known amounts of the YFV to produce samples to be used in the assay validation. The RT-qPCR workflow was performed on the Flow classic solution (Roche). Nucleic acids were extracted from 500ul of the sample. An in-vitro transcribed random RNA sequence, which is not found in nature, was spiked into plasmas during the nucleic acids extraction to function as process control. Primers/probes were obtained from the literature. YFV RNA and the control RNA were assessed by multiplex RT-qPCR. The assay's limit of detection was determined by probit regression analysis of the results obtained from a viral serial dilution from ~5000 to ~0.5 copies/mL (12 replicates of each concentration). To investigate assay's precision near the LOD three samples with ~77, ~7 and ~0.7 copies/mL of the virus were evaluated using the CLSI EP12-A2 method during three days (24 replicates of each concentration). The accuracy was evaluated using a spike recovery strategy: 96 negatives and 25 positives spiked samples were prepared and tested (viral loads in positive samples ranged from 19625 to 38 copies/mL). The total, positive and negative agreements between the expected and obtained results were evaluated. Cross-reaction with Zika, dengue and chikungunya viruses was tested. **Results:** Thirteen out of 15 quality control materials positive for YFV by MAC-ELISA tested positive for YFV RT-QPCR, and their pooling yielded 196.259 viral copies/mL. The observed limit of detection for YFV RT-qPCR was 34 copies/mL (95%CI 18-103 copies/mL). Samples ~77, ~7 and ~0.7 copies/mL returned positive results in 24/24 (100%), 16/24 (66%) and 0/24 (0%) of the tested instances, respectively. The total, positive and negative agreements between expected and observed results were 100% (95%CI 96.9-100%), 100% (95%CI 86.7-100%) and 100% (95%CI 96.2-100%). Cross-reaction with Zika, dengue and chikungunya viruses were not observed. **Conclusion:** The proposed RT-PCR method for detection of YFV is highly sensitive, the assay showed the limit of detection below 50 copies/mL. Acceptable precisions were observed for positive (>77 copies/mL) and negative results (>0.7 copies/mL). The agreement between expected and observed results were complete, and the workflow can process 96 tests in 5 hours.

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Identification of UTI pathogens using an open array platform on the QuantStudio 12K Flex system

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Background: Increasing multidrug resistance in uropathogens is leading to high recurrence rates for UTI's and has become a global challenge for antibiotic treatment regimens. It is extremely important to promptly and accurately identify the causative uropathogens for effective UTI management. We have custom designed an open array for rapid identification of 17 uropathogens using real time PCR technologies. The design of our urinary tract infection pathogen panel open array (UTI pathogen panel) allows testing of 48 urine samples for 17 targeted genes within five hours. DNA is extracted directly from urine samples and amplified on the Thermofisher QuantStudio 12k Flex open array system for detection of the following uropathogens; *Acinetobacter baumannii, Klebsiella pneumonia, Citrobacter freundii, Morganella morganii, Enterobacter aerogenes, Proteus mirabilis, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella oxytoca, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Streptococcus agalactiae* and *Candida albicans*. **Objectives:** To evaluate the UTI pathogen panel for its analytical performance characteristics and utility for patients at Accureference Medical laboratories. The real time PCR assay on the open array was compared with the currently performed traditional microbiology techniques and its performance characteristics were evaluated. **Methods:** A total of 124 urine specimens submitted for detection of UTI's by standard microbiology techniques were used for correlation studies using our UTI pathogen panel. Extraction of nucleic acids directly from urine specimens was performed using the Thermofisher MagMAX Multi sample Ultra kit on the Magmax automated extractor. Identification of uropathogens was performed on the QuantStudio 12k flex using an open array system. **Results:** Urine specimens (n=124) submitted to the microbiology laboratory for culture were tested in parallel for the presence of uropathogens using our UTI pathogen panel. A total of 90/124 specimens were identified as positive for uropathogens using the UTI panel whereas only 75 of these specimens were resulted as positive for any uropathogen by microbiology. There was 100% concordance with culture results for these 75 specimens but in 22/75 specimens (29%), at least one additional pathogen undetected by culture was identified using the UTI panel. The most frequent organisms identified in the positive specimens were *E.coli* followed by *Klebsiella pneumonia* and *Enterococcus faecalis*. Analytical sensitiv-

ity of PCR reactions for detection of the pathogens was determined by making standard curves on bacterial isolates and appropriate cut-off values were applied to correlate with bacterial loads of 10^3 colony forming units (CFU) per ml. The panel showed 100% specificity in identification of uropathogens in our studies. **Conclusions:** The UTI pathogen panel offers the advantage of identifying the cause of UTI within hours and is more sensitive than traditional microbiology methods. The panel helps to reduce the turnaround time for identification of slow growing and fastidious UTI pathogens. The molecular based semi quantitative UTI pathogen panel is a good alternative to traditional microbiology methods for sensitive and specific detection of uropathogens.

B-134

Respinning positive anti-HIV samples and retesting in duplicate - is it necessary?

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Background: Many immunoassay manufacturers recommend rerunning initial positive samples in duplicate after recentrifugation before deciding on the final result. This increases reagent usage and delays reporting. This study was designed to examine the outcome of following this practice over 6 years for the Roche Cobas Anti-HIV assay. **Methods:** Anonymised records of all repeat testing of anti-HIV analysis performed on a Roche Diagnostics e601 immunoassay analyser between 2012 and 2017 were examined in Microsoft Excel and Access. Only serum (SST II) samples are accepted for testing - all are initially spun for 4 min at 1900 g before immediate testing. Repeat testing in duplicate of initial positive samples (cutoff-index COI >=0.9) following respinning at 10 min at 4020g was performed. Any changes in result classification due to duplicate retesting was noted. **Results:** 52828 requests were received of which 98% were non-reactive. Of the remaining 960 which underwent duplicate retesting after recentrifugation, 27 were reclassified as non-reactive. The highest initial COI (iCOI) amongst these cases was 2.44 with a drop of 2.098 after respinning. The median drop in COI was 1.261. Limiting respinning and duplicate retesting to samples with iCOI 0.9-10 or 0.9-5 would reduce sample retesting by 754 and 767 cases respectively while continuing to identify the original 27 non-reactive cases. **Conclusion:** Duplicate retesting of all initially reactive anti-HIV samples is a wasteful practice. Only 2.8% of all initially reactive samples were reported as non-reactive after duplicate testing and all had low COIs. By limiting retesting to samples with COIs < 10, the number of samples undergoing retesting can be reduced by 79%.

Wednesday, August 1, 2018

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

Lipids/Lipoproteins

B-135

Sphingolipidomic Analysis Reveals Decreased Circulating Sphingomyelins and Ceramides in Sickle Cell Disease Patients

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Background: This study aimed to identify levels of C16-C24 sphingomyelin (CerP-Cho) and C16-C24 ceramide (CER) in serum obtained from SCD patients and controls. Circulating levels of neutral sphingomyelinase activity (N-SMase), ceramide-1-phosphate (C1P), sphingosine-1-phosphate (S1P) were also determined. **Methods:** Blood was collected from hemoglobin (Hb)A volunteers and homozygous HbSS patients. Serum levels of C16-C24 CerPCho and C16-C24 CER were determined by an optimized multiple reaction monitoring method using ultra fast-liquid chromatography coupled with tandem mass spectrometry. Serum activity of N-SMase was assayed by standard kit methods, C1P and S1P levels were determined by enzyme-linked immunosorbent assay. **Results:** A significant decrease was observed in serum levels of C18-C24 CerPCho and very-long-chain C22-C24 CERs in SCD patients compared to controls. A significant positive correlation was found between serum total cholesterol levels and C18-C24 CerPCho, C22-C24 CERs in SCD patients. Patients with SCD had significantly elevated serum activity of N-SMase, increased circulating levels of C1P and S1P compared to controls. **Conclusions**: Future studies are needed to understand the role of decreased CerPCho and CERs in the pathophysiology of SCD.

B-136

The effect of obesity, vitamin D level and vitamin D receptor *Fok I* single nucleotide polymorphism on serum lipid profile in children and adolescents in West China

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Background: The most of obesity could lead to dyslipidemia. Obesity closely correlates with vitamin D and vitamin D receptor (VDR). The objective of this study is to reveal the effects of obesity, serum vitamin D level and VDR *Fok I* genotype on serum lipid profile of children and adolescents in west China. **Methods:**452 children and adolescents were recruited from West China Second University Hospital to participate in this cross-section study. All the participants were divided into two groups -- obese group and non-obese group according to the body mass index (BMI). Serum vitamin D level, serum lipid level and VDR *Fok I* gene polymorphism were detected in the laboratory. Based on the level of Serum vitamin D, all subjects were divided into three group, vitamin D normal group, vitamin D insufficiency group and vitamin D deficiency group. All children and adolescents were classified into TT genotype and C allele carriers on the basis of the different expression of VDR *Fok I* gene. The impact of obesity, vitamin D level and VDR *Fok I* genotype on lipid level was investigated by analysis of the experiment data. All data were analyzed by independent-samples T test and One-way ANOVA , and adjusted for age by covariance test. **Results:** 1. The concentrations of serum vitamin D in the obese group was lower than that in the non-obese group. The levels of serum TC, TG, HDL, LDL, Apo-A1, Apo-B, LDL/HDL, TG/HDL in the obese group were higher than those in the non-obese group (P=0.000, P=0.003, P=0.000, P=0.000, P=0.000 P=0.000, P=0.000). 2. There was no difference in genotype distribution and allele frequency of VDR *Fok I* site between Obese and non-obese group. 3. In all children and adolescents, there was no difference in serum vitamin D level and lipid profile between C allele carriers and TT genotype in non-obese group. However, in obese group, the C allele carriers had much lower concentrations of TC, TG, Apo-B, TC/TG, LDL/HDL, TG/HDL than TT genotype (P=0.000, P=0.017, P =0.000, P =0.009, P=0.033, P =0.020). 4. The concentration of HDL-C and Apo-A1 in the vitamin D deficiency group was significantly higher compared with the insufficiency and normal group (P=0.007, P=0.001; P=0.013, P=0.002). Moreover, the vitamin D insufficiency and deficiency group had higher concentration of TC and LDL-C compared with vitamin D normal group (P=0.025, P=0.012; P=0.044, P=0.032).5. In non-obese group, C allele carriers had higher TC, HDL, Apo-A1 in vitamin D deficiency group compared with TT genotype (P=0.039, P=0.025, P=0.009). In obese group, C allele carriers had lower concentrations of TC, TG, Apo-B, TC/HDL, LDL/HDL and TG/HDL in vitamin D deficiency group than TT genotype (P=0.009, P=0.011, P=0.001, P=0.000, P=0.007, P=0.008). **Conclusion:** The level of lipid is influenced by mutation of VDR Fok I gene, the level of vitamin D and obesity in children and adolescents in west China. The effect of the mutation of VDR genotype could be reinforced when the subjects had low concentration of vitamin D. The molecular mechanism of VDR genotype effect on lipid level requires a further research.

B-137

Correlation of two Lipoprotein(a) assays, Lipoprotein(a) particle concentration and Lipoprotein(a) cholesterol assay

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Background: Lipoprotein(a) [Lp(a)] is consisted of modified LDL particle and an apolipoprotein(a) [apo(a)]. It is considered as a risk factor for cardiovascular event for many years, but most of the methods used in clinical laboratory are affected by apo(a) size heterogeneity and there are problems with size-sensitive results as well as standardization between assays. So we investigate the correlation and their characteristics two Lp(a) assays Tina-quant Lipoprotein(a) Gen.2 [LA(2)] (Roche Diagnostics GmbH) for Lp(a) particle concentration and Tina-quant Lipoprotein (a) (Latex) [LA(1)] for Lp(a) cholesterol assay. **Methods:** Total 400 consecutive clinical samples submitted for Lp(a) test from March to September, 2015, were studied. All the analysis were done with cobas c 501 analyzer (Roche Diagnostics GmbH) and more than 30 mg/dL of LA(1) and more than 75 nmol/L of LA(2) were considered as the clinical cut-off for the high risk group for atherosclerosis. For the correlation analysis of the results from LA(2) with LA(1), conversion factor, 0.4167 in the manufacture's insert was used. We performed the correlation analysis, kappa value, Fisher's exact test, and Student's t-test (when p values < 0.05 considered as statistically significant). All the statistical analyses were done with Analyse-it for Microsoft Excel (Ver. 4.65). **Results:** The correlation between two assays were not bad (Pearson's r = 0.7). And LA(1) results of 391 samples were larger than LA(2) converted, which showed statistical significance (P<0.00). Sixty-one samples among 400 samples were classified as high risk group in LA(2) and 114 samples were classified as high risk group in LA(1). The kappa value of high risk group of two assays was 0.62 (95% confidence interval 0.53-0.70). **Conclusions:** We observed that LA(1) results tended to be overestimated compared to LA(2) ($P<0.05$). And there were discrepancy between two assays for classifying high risk group for atherosclerosis. There are not enough clinical studies based on the difference between particle concentration and mass analysis. Also, standardization of Lp(a) is still far, many clinical guideline are still based on the older assay affected by apo(a) size heterogeneity and ethnic difference. So we suggested that the standardization should be done as soon as possible. And further studies including the establishment of the reference interval in the various ethnic group and clinical studies based on Lp(a) particle concentration are required.

B-138

Performance characteristics of a novel direct assay for small, dense LDL cholesterol on Roche and Beckman chemistry analyzers

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Background: Small, dense LDL (sd LDL) is a highly atherogenic lipoprotein, and many clinical studies have strengthened the relationship between sd LDL-C level and CHD. We have developed a simple and fully-automated homogeneous assay for quantification of sd LDL-C (s LDL-EX"SEIKEN"). The assay received 510(k) clearance in August, 2017 based upon the performance data obtained on the Roche / Hitachi 917 analyzer. We further evaluated the assay performance on additional systems; Roche cobas c501 and Beckman AU5800. **Method:** Precision, linearity, limit of quantitation (LOQ) studies and a comparison study to Hitachi 917 were performed according to CLSI approved guideline EP05-A2, EP06-A, EP07-A2, EP17-A2 and EP09-A3, respectively. Equivalency between serum and plasma samples was evaluated using 40 paired samples. Reagent open vial stability was evaluated for up to 5 weeks. A calibration frequency was determined from results of weekly measurements up to 3 weeks by using the calibration curve obtained at the initial time point. **Result:** Within-laboratory %CV of the assay was 1.3-2.3% on Roche cobas c501, and

1.8-3.3% on Beckman AU5800. Linear assay range was confirmed up to 100 mg/dL for both analyzers. LOQ was established as 1.6 mg/dL and 0.6 mg/dL on Roche cobas c501 and Beckman AU5800, respectively, ensuring the lower measurement limit of the assay (4 mg/dL). Results with patient samples were substantially equivalent among Roche/Hitachi 917, Roche cobas c501 and Beckman AU 5800 (r>0.95). No sample matrix related bias was observed in a study with 40 paired serum and plasma samples. Reagent remained stable and functional in opened vials for 4 weeks and the calibration was stable for two weeks on both analyzers, comparably to Hitachi 917. **Conclusion:** The analytical performance of sd LDL-C assay was verified on Roche cobas series and Beckman AU series. These instrument families will be added on the list for this FDA-approved assay to lead expanded use in clinical laboratories.

B-139

Performance Evaluation of the Atellica CH Chol_2, D-HDL, DLDL, and Trig Assays

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Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH Chol_2, D-HDL, DLDL, and Trig Assays on the Atellica CH Analyzer. Measurement of these assays is used in assessing the risk of developing heart disease and monitoring patients with existing heart disease. The Atellica CH D-HDL Assay (D-HDL) and the Atellica CH DLDL Assay (DLDL) involve two reactions. The first reaction uses cholesterol esterase and cholesterol oxidase to isolate HDL or LDL cholesterol. The second reaction uses cholesterol esterase, cholesterol oxidase, and peroxidase—along with hydrogen peroxide, 4-aminoantipyrine, and a hydrogen peroxide-detecting molecule—to form a quinoneimine complex in a Trinder endpoint reaction. The absorbance of the complex is measured, and the intensity is directly proportional to the amount of cholesterol in the sample. The Atellica CH Chol_2 Assay (Chol_2) uses the same methodology; however, it involves only the second reaction. The Atellica CH Trig Assay (Trig) uses a similar methodology: the molecule is broken down to glycerol and fatty acids, eventually producing hydrogen peroxide. When the hydrogen peroxide is combined with 4-aminophenazone, 4-chlorophenol, and peroxidase, it produces a quinoneimine-dye complex. **Method:** Performance testing included precision and accuracy. Assay precision was evaluated using the 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 patient-sample results compared to results from the ADVIA® 1800 Clinical Chemistry System. **Results:** For Chol_2, within-lab precision ranged from 0.8-1.3% CV in serum/plasma samples. For D-HDL, within-lab precision ranged from 0.7-2.0% CV in serum/plasma samples. For DLDL, within-lab precision ranged from 1.0-2.3% CV in serum/plasma samples. For Trig, within-lab precision ranged from 1.0-2.5% CV in serum/plasma samples. The Chol_2 serum method comparison study yielded a regression equation of y = 0.97x + 1 mg/dL with r = 0.997, versus the ADVIA 1800 Chol_2 Assay. The D-HDL serum method comparison study yielded a regression equation of y = 0.97x + 1.4 mg/dL with r = 0.999, versus the ADVIA 1800 D-HDL Assay. The DLDL serum method comparison study yielded a regression equation of y = 0.99x - 0.8 mg/dL with r = 1.000, versus the ADVIA 1800 DLDL Assay. The Trig serum method comparison study yielded a regression equation of y = 0.98x + 0.5 mg/dL with r = 0.999, versus the ADVIA 1800 TRIG_2 Assay. **Conclusions:** The Atellica CH Chol_2, D-HDL, DLDL, and Trig Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

B-140

Early Postoperative Changes of Sphingomyelins and Ceramides After Laparoscopic Sleeve Gastrectomy

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Background: This study aimed to determine early postoperative changes of serum sphingomyelin (SM) and ceramide (CER) species following laparoscopic sleeve gastrectomy (LSG). **Methods:** Twenty obese patients [mean body mass index (BMI) 45,64 ± 6,10 kg/m^2] underwent LSG and normal weight control patients (mean BMI 31,51 ± 6,21 kg/m^2) underwent laparoscopic cholecystectomy. Fasting blood samples were collected prior to surgery, at day 1 and day 30 after surgery. Circulating levels of C16-C24 SMs, C16-C24 CERs and sphingosine-1-phosphate (S1P) were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Ceramide-1-phosphate (C1P) levels were determined by enzyme-linked immunosorbent assay (ELISA). Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods. Insulin sensitivity was evaluated using homeostatic model assessment for insulin resistance (HOMA IR). **Results:** A significant decrease was observed in serum levels of very-long-chain C24 SM, very-long-chain C22-C24 CERs and C1P in LSG patients after postoperation day 1 and day 30 compared to preoperation levels. At 30 days postsurgery, BMI was reduced by 11 %, fasting triglycerides were significantly decreased, and insulin sensitivity was increased compared to presurgery values. A significant positive correlation was found between HOMA-IR and serum levels of C22-C24 CERs in LSG patients. **Conclusions**: We conclude that very long chain CERs may mediate improved insulin sensitivity after LSG.

B-141

Performance of Diazyme Laboratories, Inc. Lp(a) Assay Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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Background: The Diazyme Laboratories, Inc. Lp(a) Assay is intended as a latex particle enhanced immunoturbidimetric assay for the in vitro quantitative determination of lipoprotein(a) concentration in serum or EDTA plasma. Lipoprotein (a) is a cholesterol-rich lipoprotein particle found in human serum. There is substantial evidence linking lipoprotein (a) excess to a high risk for premature coronary heart disease (CHD), increased risk of myocardial infraction (MI), stroke, restenosis after angioplasty (PTCA) and coronary bypass procedures. The Diazyme Lipoprotein (a) Assay is based on a latex enhanced immunoturbidimetric methodology. Lp(a) in the sample binds to specific anti-Lp(a) antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically at 700 nm and is proportional to the amount of Lp(a) in the sample. **Methods:** The performance of the Diazyme Lp(a) assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System was assessed on the VITROS MicroTip assay processing side of the MicroImmunoassay Center using 4.0 uL patient samples and the Diazyme Lp(a) reagents on the VITROS 4600/5600 Systems compared to predicate device Roche Hitachi 917 analyzer following CLSI: EP9-A2 guidelines. **Results:** The accuracy of Diazyme Lp(a) assay was evaluated with 80 patient serum samples (0.57 -108.34 mg/dL) on patients on the VITROS 4600 and VITROS 5600 System. Both showed excellent correlation with the Roche Hitachi 917. VITROS 4600 System R^2 value of 0.9918 with a slope= 0.9737, and y-intercept of +2.495. VITROS® 5600 System R^2 value of 0.9935 with a slope= 0.969, and y-intercept of -0.3492. A 20-day precision study conducted on the VITROS 4600 system at mean Lp(a) concentrations of 16.3 mg/dL and 47.9 mg/dL resulted in within-laboratory percent coefficient of variation (%CV) of 3.02 % and 1.74% respectively, for the VITROS 4600 System and 2.94% and 1.13% respectively, for the VITROS 5600 System. . The Limit of Quantification (LoQ) check for the VITROS® 4600 and VITROS 5600 Systems was found to be ≤5.44 mg/dL. At 17 mg/dL common interfering endogenous substances of ascorbic acid 10 mM, bilirubin 40 mg/dL, conjugated bilirubin 40 mg/dL, hemoglobin 1000 mg/dL and triglycerides 1000 mg/dL showed no significant interference (≤ 10%). **Conclusion:** The Diazyme Lp(a) assay run on the VITROS 4600 and VITROS 5600 Systems demonstrated excellent correlation with the Roche Hitachi 917 Clinical Chemistry Analyzer, exceptional precision, and low-end sensitivity. Additionally, the assay was free from interference by endogenous substances at clinically relevant Lp(a) concentrations.

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Postprandial GLP-1 response to a high-fat meal is blunted in obese adolescents with insulin resistance and metabolic dyslipidemia

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Background: Obesity and insulin resistance are becoming increasingly prevalent in adolescents and are commonly associated with dyslipidemia. Postprandial, rather than fasting, dyslipidemia independently predicts cardiovascular disease risk and is characterized by intestinal triglyceride-rich lipoprotein (TRL) overproduction. Co-secreted intestinal peptides, glucagon-like peptide 1 (GLP-1) and 2 (GLP-2), have been shown to attenuate and paradoxically augment intestinal TRL output, respectively, in both animal and human studies. We hypothesize that postprandial GLP-1 and GLP-2 responses are

altered in obese adolescents with insulin resistance and/or postprandial dyslipidemia. **Methods:** Normal weight (n=15; 8M/7F) and obese (n=15; 8M/7F) adolescents underwent an oral fat tolerance test (83% kcal from fat). Blood was collected at fasting and 1, 2, 4, and 6 hours following meal ingestion. The lipid profile, glucose, and insulin were measured on the Abbott ARCHITECT ci4100 analyzer. GLP-1 (active and total) and GLP-2 were measured by ELISA. The area under the curve (AUC) and incremental AUC (iAUC) of the postprandial profile was calculated using the trapezoidal method, with 0 and fasting values used as the baseline, respectively. Data were log-transformed prior to analysis if not normally distributed. AUC and iAUC were compared using independent samples t-test. Two-way, mixed analysis of variance (ANOVA) with time as a repeated measure, was used to test differences between each postpandial time point and fasting within each group, as well as test differences between groups at fasting and each postprandial time point. **Results:** Postprandial active GLP-1 (area under the curve (AUC), incremental AUC (iAUC)) and total GLP-1 (iAUC) were significantly lower in obese compared to normal weight adolescents, suggesting a blunted GLP-1 response to fat ingestion. However, when dividing the obese cohort by the presence of insulin resistance, postprandial active GLP-1 (AUC, iAUC, 2hour) and total GLP-1 (iAUC) were significantly lower only in obese adolescents with insulin resistance compared to normal weight. Furthermore, only obese adolescents with insulin resistance lacked a significant postprandial rise in active and total GLP-1. When dividing the obese cohort by the presence of postprandial dyslipidemia, postprandial active GLP-1 (AUC, 6 hour) was significantly lower only in obese subjects with postprandial dyslipidemia compared to normal weight adolescents. Postprandial GLP-2 tended to be higher in obese subjects, although this difference was not significant, even when obese adolescents with insulin resistant and postprandial dyslipidemia were each examined separately. However, a significant postprandial rise in GLP-2 was absent from obese subjects with insulin resistance and postprandial dyslipidemia, suggesting a blunted postprandial GLP-2 response in obese subjects with these metabolic conditions. **Conclusion:** The postprandial GLP-1 and GLP-2 response to a high-fat drink appears to be blunted in obese adolescents, but only in the presence of insulin resistance or postprandial dyslipidemia. However, it remains unknown if a blunted postprandial gut peptide response is a cause or consequence of the progression of these metabolic conditions in an obese state.

B-143

Extreme eruptive Xanthomas Associated with Severe Hypertriglyceridemia, Diabetes Mellitus and Hypothyroidism: a Case Report

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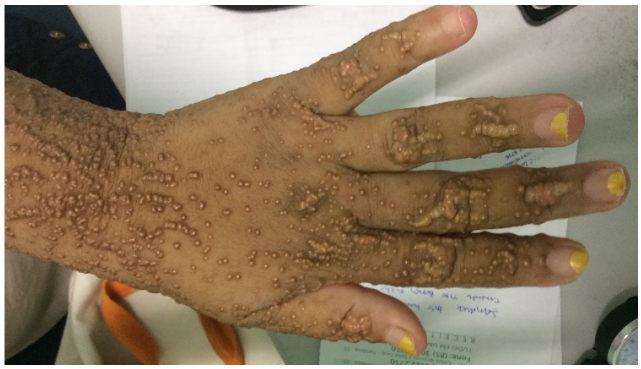
Case Report: A 37-year-old woman, with type 2 diabetes mellitus, presented with a pruritic skin rash of 3-months' duration. She had a history of total thyroidectomy for papillary thyroid cancer. Physical examination: multiple red-to-yellow papules, disseminated on both upper and lower extremities. A yellow deposit on the conjunctiva, and a yellowish discoloration of the distal ungeal portions were observed. Fasting blood sample: total cholesterol level of 1,252 mg/dL, a high density lipoprotein cholesterol of 34 mg/dL, a triglyceride level of 8,229 mg/dL, a glucose of 371 mg/dL (20.5 mmol/L), a glycated hemoglobin (HbA1c) of 11%, and a TSH of 41 mUI/mL. Punch biopsy of the skin lesions: lipid deposits and confirmed the diagnosis of Eruptive Xanthoma. Started on a 1,200-kcal diet, a full insulin regimen and fenofibrate 160 mg. The metformin dose of 2 g per day was maintained and the levothyroxin dose was uptitrated to 175 mcg. Six moths later, the diabetes and hypothyroidism were better controlled (HbA1c: 7,3% and TSH: 2,85 mUI/mL). The triglyceride levels dropped to 137 mg/dL, and the skin lesions were resolving.



B-144

Polymorphic frequency of *APOE* gene from a clinical laboratory database

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Background: The apolipoprotein E gene (*APOE*) has an important role in lipoprotein metabolism. The study of the polymorphism variability allows in the determination of predisposition of some diseases such as Alzheimer's and cardiovascular diseases. Due to its clinical importance, the allelic distribution of this gene has been studied in different populations and ethnical groups in order to establish the diseases´ genetic profile. The objective of this study is to perform a yearlong (2017) data analysis to determine the allelic and genotypical frequencies of the *APOE* gene in samples tested in a Brazilian Clinical Laboratory. **Methods:** The data found were provided by ShiftLis software, used by our laboratory, and we conducted a descriptive analysis. The studied samples were processed by two distinctive methodologies, both using the same primers set described in the literature. The first of the two methodologies was a manual DNA extraction that utilizes Chelex®-100 Resin (BioRad), and processed in StepOne™ Real-time PCR System (Applied Biosystems) using Maxima SYBR Green/ROX qPCR Master Mix (Applied Biosystems). After the inicial technical validation there was a change in the methodology and we started using an automated DNA extraction MagNA Pure 96 System (Roche Life Science) and processed in Roche LightCycler®480 II (Roche Life Science) with LightCycler® 480 SYBR Green (Roche Life Science). **Results:** A total of 350 clinical samples were obtained in a period of one year, those being: 255 (72,9%) females, ages 6-86 (43.41±14.68) and 95 (27,1%) males, ages 8-83 (44.20±15.51). The analyzed samples came from nine different Brazilian cities: Manaus (56,5%), Brasilia (28%), Salvador (12%), Sao Jose dos Campos (1.4%), Palmas (0.9%), Campo Grande, Valparaiso, Ribeirao Preto and Campinas (0.3%). The highest allelic frequency found was E3 (80.3%), followed by E4 (15.1%) and finally E2 (4.6%). The largest genotypical group found was E3/E3 (64,9%), E3/E4 (24,0%), E2/E3 (6.9%), E4/E4 (2.3%), E2/E4 (1.7%) and the smallest one was E2/E2 (0.3%). The allelic frequencies obtained in this study were similar to the data of other Brazilian regions previously studied E3 (71.78 - 80.49%), E4 (13.41 - 22.88%) and E2 (2.73 - 6.63%) and from a recent worldwide study E3 (48 - 91%), E4 (6 - 40%) and E2 (<3 - >9%). **Conclusion:** This was the first study to analyze the polymorphic frequency of the *APOE* gene in a clinical laboratory, without the restriction of ethnical groups, ages, gender or previous known conditions, and also the first one to amass the results of six new Brazilian cities that haven´t been studied prior to this occasion, thus contributing to further genetic population studies.

B-145

Comparison of calculated and directly measured low-density lipoprotein cholesterol at different triglyceride levels

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Background: Low-density lipoprotein cholesterol (LDL-C) is one of the major risk factors for coronary heart disease (CHD). LDL-C concentration is used in the National Cholesterol Education Program (NCEP) guideline for CHD risk categorization and therapeutic target. Therefore, accurate measurement of LDL-C is important for CHD patient management. Currently, calculation of LDL-C by the Friedewald equation is the most widely used laboratory method to determine LDL-C concentration. However, requirement of fasting and failure to accurately quantify LDL-C

when triglyceride (TG) greater than 400mg/dL limit its utilization. The aim of this study is to compare the serum LDL-C concentration measured by homogeneous enzymatic method ($LDL\text{-}C_D$) and LDL-C concentration calculated by Friedewald equation ($LDL\text{-}C_F$) and the novel equation based on the 180-cell table developed by the Johns Hopkins group ($LDL\text{-}C_{180}$). **Methods:** The lipid profile data were randomly retrieved from 257 patient serum samples. The determination of LDL-C concentrations by enzymatic method was performed on Beckman AU5800 analyzer. Lipid profiles (total cholesterol, HDL-C, and TG) measured by AU5800 were used to calculate LDL-C by the Friedewald equation (total cholesterol-HDL-TG/5) and the novel equation (total cholesterol -HDL-TG/adjustable factor based on the 180-cell table). Method comparison was done by EP Evaluator Software. **Results:** Of the 257 samples, 197 (76.7%) have TG level less than 200mg/dL, 34 (13.2%) have TG level between 200 and 400 mg/dL, and 25 (9.7%) have TG level greater than 400mg/dL. The overall comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_F$ showed the slope was 1.108 with intercept of -14.30, correlation coefficient of 0.8648, and the mean bias of -3.26. The overall comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_{180}$ showed the slope was 0.978 with intercept of 16.28, correlation coefficient of 0.9428 and the mean bias of 6.34. For the 197 samples with TG level less than 200 mg/dL, comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_F$ showed the slope was 0.943 with intercept of 6.59, correlation coefficient of 0.9353, and the mean bias of 0.69; comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_{180}$ showed the slope was 0.923 with intercept of 9.58, correlation coefficient of 0.9429, and the mean bias of 1.69. For the 34 samples with TG level between 200 and 400 mg/dL, comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_F$ showed the slope was 1.070 with intercept of -14.11, correlation coefficient of 0.8813, and the mean bias of -6.65; comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_{180}$ showed the slope was 0.936 with intercept of 12.55, correlation coefficient of 0.8827, and the mean bias of 5.82. For the 25 samples with TG level greater than 400 mg/dL, comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_F$ showed the slope was 1.523 with intercept of -78.89, correlation coefficient of 0.7913, and the mean bias of -29.74; comparison of $LDL\text{-}C_D$ with $LDL\text{-}C_{180}$ showed the slope was 1.022 with intercept of 14.16, correlation coefficient of 0.8851, and the mean bias of 16.24. **Conclusions:** These data demonstrate that LDL-C concentration measured by enzymatic method has the best correlation and least bias with both calculated LDL-C when TG level is less than 200mg/dL. When TG level is greater than 400 mg/dL, $LDL\text{-}C_D$ has a better correlation with $LDL\text{-}C_{180}$ than with $LDL\text{-}C_F$.

B-146

Dyslipidemia and associated cardiovascular risk factors among young university students of Nepal

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Background: Cardiovascular diseases (CVDs) are one of the main causes of morbidity and mortality worldwide, atherosclerosis being the principal underlying cause of CVDs. Dyslipidemia is the most important risk factor for atherosclerosis. Although CVDs are not observed in childhood, cardiac risk factors such as dyslipidemia are present in children and they remain silent until adulthood. Cardiovascular risk factors are on the rise in the Nepalese population and recent observation has shown a significant elevation of risk factors in the youth population. The purpose of this study was to assess dyslipidemia and associated cardiovascular risk factors among young university students of Nepal **Methods:** A cross sectional study was carried out in Institute of Medicine (IOM), Tribhuwan University Teaching Hospital. The study was conducted from 20th February 2017 to 20th July 2017.The study population encompassed 280 undergraduate students aged 17-24 (156 males and 124 females) during the time frame. Recruitment was done randomly and eligible participants were selected if they were healthy, physically active and taking no medications known to influence lipid metabolism. An interview-based questionnaire was designed and information about age, sex, smoking and alcohol consumption was collected. Body mass index and waist-to-hip ratio of all participants were calculated. Venous blood samples were obtained from the antecubital vein in suitable vacutainers after 12 hours overnight fasting. Triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL) were analyzed enzymatically and the low-density lipoprotein cholesterol (LDL) was calculated using Friedwald's formula. All the estimations were done using the autoanalyser (BT 1500) **Results:** Overall, dyslipidemia was seen as Hypercholesterolemia in 31 (11.1%), elevated low-density lipoprotein in 34 (12.1%), low high-density lipoprotein in 95 (33.9%) and hypertriglyceridemia in 39 (13.9%). Risk factors found to be significantly associated with Hypercholesterolemia were a history of smoking ($p< 0.01$, OR: 3.6) and binge drinking ($p < 0.001$, OR: 8). Sex ($p < 0.05$, OR: 0.41), binge drinking ($p <0.001$, OR: 5.1) and smoking ($p<0.001$, OR: 11.3) were found to be significantly associated with elevated LDL. Sex ($p <0.05$, OR: 0.45), Binge drinking ($p<0.05$, OR: 1.67) and smoking ($p<0.05$, OR: 3.5) were significantly associated with hypertriglyceridemia. There was no statistically significant association between risk factors and the low HDL. Multivariate Logistic regression analyses showed that current smoking and binge drinking were significant predictors of Hypercholesterolemia and elevated LDL. In females, only the serum LDL level was significantly correlated with BMI however, serum TC and LDL levels both significantly correlated with WHR. Meanwhile, in the case of males, all TC, TG, LDL, HDL levels significantly correlated with BMI and WHR. **Conclusion:** The prevalence of dyslipidemia was high in young Nepalese university students (17-24 years). Early detection of dyslipidemia and long-term prevention of cardiovascular disease by controlling the risk factors should begin in youth. Increasing student awareness of importance of controlling alcohol consumption, quitting smoking and increasing physical activity is of paramount importance to reduce dyslipidemia prevalence and prevent cardiovascular disease.

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Determination of serum triglyceride by Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry

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Background: There are some reference methods to determine serum total glycerol and triglyceride all by isotope dilution gas chromatography mass spectrometry which are time consuming and complicated. A need exists for a simple reference method that can be easily adopted to verify the accuracy of serum triglyceride measurements, especially with different measurement principles. Just as serum triglyceride concentrations are generally determined from total glycerol with or without a subtraction of free glycerol. So candidate reference methods involving isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) for total glycerol and free glycerol were established. The triglyceride concentration was the difference between total glycerol and free glycerol. **Methods:** An isotopically labeled internal standard, [$^{13}C_3$]-glycerol, was added to serum, protein precipitation, and derivatization by benzoyl chloride to prepare samples for LC/MS/MS analysis using electrospray for ionization (ESI). For total glycerol, hydrolysis was conducted after adding the internal standard. For separation, a Nova-Pak C18 column was used with a mobile phase consisting of 10 mmol/L ammonium formate in water-acetonitrile (20:80 by volume) for positive ions. The quantitative ion transitions of $[M+NH4]^+$ at m/z 422.2→283.2 and m/z 425.2→286.2 were monitored for glycerol and [$^{13}C_3$]-glycerol, respectively. The qualitative ion transitions were at m/z 422.2→105.1 and m/z 425.2→108.1, respectively. The method was calibrated with linear regression using five-point calibration curves. **Results:** The correlation coefficients between the peak area ratios and glycerol concentrations were 0.9999 and higher. The within-run coefficients of variation (*CV*) for serum total glycerol analysis averaged 0.52% (ranged 0.3%~1.02%) and the total *CV* 0.73% (0.49%~1.27%). Results on certified reference materials (SRM 909b Level I and Level II, SRM 1951b Level I and Level II, SRM 909c, GBW 09146 and GBW 09147) showed an averaged bias of 0.32% (0%~0.97%). **Conclusion: I**sotope dilution LC/MS/MS method for serum triglyceride has been developed. This method was been used in international laboratory comparison including RELA (IFCC) and Cholesterol Reference Method Laboratory Network(CRMLN,US. CDC). Results showed that this ID-LC/MS/MS method was well-characterized for serum glycerides with a theoretically sound approach, demonstrated good accuracy and precision, and low susceptibility to interferences qualifies as a candidate reference method. Use of this reference method as an accuracy base may reduce the apparent biases in routine methods along with the high interlaboratory imprecision.

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Developing a modified low-density lipoprotein (M-LDL-C) Friedewald's equation as a substitute for direct LDL-c measure in the Ghanaian Setting

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Background: An elevated concentration of low density lipoprotein cholesterol (LDL-c) is a well-known atherogenic risk factor with a high predictive value for coronary heart disease. Though a number of homogenous assay are available for estimation of LDL-c, use of calculated LDL-c by Friedewald's formula (FF) is common in Ghanaian laboratories for logistic reasons. Several novel formulae (Martin's, Vojovic's and Anandaraja's formula) have been reported to outperform the Friedewald formula. This study therefore validated existing formulae and derived a more accurate formula to determine LDL-c in a Ghanaian population.

Methods: We estimated 1518 lipid profiles from the outpatient department of the Cardiothoracic Centre of the Korle-bu Teaching Hospital. We evaluated three formulae (Friedewald, Anandaraji, and Martin's) and compared these to direct measurement of LDL-c across triglyceride (TG) high density lipoprotein (HDL-c) and total cholesterol (TC) ranges using a reagent kit obtained from Human Diagnostic Worldwide, Germany and URIT 8210 automatic chemistry analyzer. Using values of lipoproteins from the initial measurements in our population, a new modified Friedewald's LDL-c (M-LDL-C) equation was derived by replacing the term 2.2 with 4.0. Receiver operator characteristic (ROC) and linear regression were performed. Data was analysed using STATA version 12.0 **Results:** The mean LDL-c concentration measured by enzyme-based direct homogenous assay (D-LDL-c) and that calculated by Friedewald's formula (F-LDL-c), Martin's formula (N-LDL-c), Anandaraja's formula (A-LDL-c) and modified Friedewald's LDL-c (M-LDL-C) formula were 2.47±0.71 mmol/L, 2.76±1.05mmol/L, 2.74±1.04 mmol/L, 2.99±1.02 mmol/L and 2.97±1.08 mmol/L respectively. D-LDL-c levels were significantly lower compared to F-LDL-c, N-LDL-c, A-LDL-c and M-LDL-C ($p < 0.001$) using the Student paired t-test. The F-LDL-c equation showed a significantly strong positive correlation with A-LDL-c ($r=0.898$, $p<0.0001$), N-LDL-c ($r=0.991$, $p<0.0001$) and M-LDL-c ($r=0.989$, $p<0.0001$), but a weak positive correlation with LDL-c ($r=0.481$, $p<0.0001$). Analysis on ROC curve showed a better diagnostic accuracy for M-LDL-c (AUC=0.81) and N-LDL-C (AUC=0.81) followed by F-LDL-c (AUC=0.80) and A-LDL-c (AUC=0.77) based on a D-LDL-c cut-off >2.5mmol/L. Bland-Altman graphs showed a definite agreement between mean and differences of the calculation formulae and D-LDL-C with 95% of values lying with in±0.50 SD limits. **Conclusion:** The modified LDL-c (M-LDL-c) equation could serve as a better substitute for both D-LDL-c and F-LDL-c equation in the Ghanaian settings.

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Performance Evaluation of the Atellica CH Apolipoprotein A-1 and Apolipoprotein B Assays

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Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH **Apolipoprotein A-1 (APO A1)** and **Apolipoprotein B (APO B)** Assays on the Atellica CH Analyzer. Measurements of APO A1 and APO B are used in assessing arteriosclerosis development and the severity of coronary artery stenosis. APO A1 and APO B use a PEG-enhanced immunoturbidimetric methodology. APO A1 or APO B in the sample forms an insoluble complex with a specific antiserum, which is measured turbidimetrically. **Method:** Performance testing included precision and accuracy. Assay precision was evaluated using the 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 patient sample results compared to results from the ADVIA® 1800 Clinical Chemistry System. **Results:** For APO A1, within-lab precision ranged from 1.7-2.4% CV in serum/plasma samples. For APO B, within-lab precision ranged from 2.8-6.1% CV in serum/plasma samples. The APO A1 serum method-comparison study yielded a regression equation of $y = 1.05x - 1$ mg/dL with $r = 0.989$, versus the ADVIA 1800 APO A1 Assay. The APO B serum method comparison study yielded a regression equation of $y = 0.99x - 4$ mg/dL with $r = 0.999$, versus the ADVIA 1800 APO B Assay. **Conclusions:** The Atellica CH APO A1 and APO B Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

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LDL-cholesterol determination by measured LDL-cholesterol versus Martin equation for triglycerides levels above 400 mg/dL

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Background: The determination of the low-density lipoprotein cholesterol (LDL-C) is classically obtained by Friedewald equation. This equation assumes a fixed factor of 5 for the ratio of triglycerides to very low-density lipoprotein cholesterol. The Friedewald equation should not be used when plasma triglycerides concentration exceeds 400 mg/dL. A novel method for estimating LDL-C reported by Martin et al has advocated use of a newly derived equation to estimate LDL-C that is intended to correct for this limitation in the Friedewald calculation and improve LDL-C estimation even when triglycerides values are >400 mg/dL. The novel LDL-C is calculated using an adjustable factor determined on the basis of an individual patient's triglycerides and non-HDL-Cholesterol (non-HDL-C). The aim of this study was to compare the LDL-C results using the Martin equation versus directly measured LDL-C for triglycerides values above 400 mg/dL. **Methods:** The results of 319 directly measured LDL-C previously released in a laboratory routine were recalculated using the equation of Martin et al. The total cholesterol, HDL-C, LDL-C and triglycerides were carried out on the Roche 8000 analyzer (Roche Diagnostics GmbH, Germany) using reagents from Roche. The mean triglycerides value was 570 ± 211 mg/dL ranging from 401 to 1888 mg/dL. **Results:** The values of measured LDL-cholesterol and obtained by calculation were respectively: Mean ± SD: 132 ± 49 mg/dL and 128 ± 44 mg/dL; Median: 129 mg/dL and 126 mg/dL; First Quartile (box-plot): 99 mg/dL and 100 mg/dL; Third Quartile (box-plot): 163 mg/dL and 155 mg/dL; The linear regression equation considering measured versus calculated LDL-C was: $y = 0.851x + 15.889$ ($R^2 = 0.8906$). **Conclusion:** The calculation of LDL-C using the Martin equation showed a good correlation with directly measured LDL-C results, a fact that encouraged us to implement in the routine standard lipid profile of our Service.

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Performance characteristics of a novel direct assay for small, dense LDL cholesterol, and results from a reference range study

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Background: Small, dense LDL (sd LDL) is a highly atherogenic lipoprotein, and many clinical studies have strengthened the relationship between sd LDL-C level and CHD. We have developed a simple and fully-automated homogeneous method for quantification of sd LDL-C (s LDL-EX"SEIKEN"). A series of basic performance studies as well as reference range study were performed using the s LDL-EX "SEIKEN" on the Roche Diagnostics Hitachi 917 analyzer. This kit received 510(k) clearance in August, 2017 **Method:** Precision, linearity, interference, limit of quantitation (LOQ) and correlation with ultracentrifugation method (UC) were performed according to CLSI approved guideline EP05-A2, EP06-A, EP07-A2, EP17-A2 and EP09-A3. Matrix comparison was carried out using 47 paired serum and plasma samples. Reference range study was conducted in accordance with EP28-A3c. Subjects were recruited from two geographical regions in the US. Based on Adult Treatment Panel III (ATPIII) guideline, subjects were partitioned by age and gender. **Result:** Within-laboratory %CV was 1.3 to 4.1%. Linear assay range was confirmed up to 100 mg/dL. Established LOQ was 1.14 mg/dL, which is below the lower measurement limit (4 mg/dL). No interference of hemoglobin, bilirubin, chyle, statins, fibrates was found against sd LDL-C values. The results were in good correlation between this kit and the recognized UC reference method (slope: 1.028 [95%CI 0.932 - 1.127], intercept: -1.38 [95%CI -5.73 - 1.80]). Age differences associated with the sd LDL-C level were significant in both genders ($p = 0.0030$ in males and $p < 0.0001$ in females). No significant difference was observed in the sd LDL-C level between males and females ($p = 0.7564$). According to the CLSI guideline, the normal range was defined as the 2.5th percentile value to the 97.5th percentile value, younger group was 12.7 to 48.3 mg/dL and older group was 12.6 to 51.7 mg/dL. **Conclusion:** The sd LDL-C assay demonstrated good analytical performance. This FDA-approved assay is promising for application in routine clinical practice as an IVD product.

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Clinical significance of measurement by novel direct assay for small, dense LDL cholesterol for CHD risk assessment

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Background: Small, dense LDL (sd LDL) is an atherogenic lipoprotein, and many clinical studies have strengthened the relationship between sd LDL-cholesterol(C) level and incident CHD. We have developed a simple and technically conventional method for quantification of sd LDL-C (s LDL-EX"SEIKEN"). The clinical cutoff value was established by Multi-Ethnic Study of Atherosclerosis (MESA) and was validated using the cohort of Atherosclerosis Risk In Community (ARIC) study. This kit received 510(k) clearance in August, 2017. **Method:** The Adult Treatment Panel III has generally selected the 75th percentile value for LDL-C as being associated with high risk of CHD. Based on this principle, the 75th percentile value was selected as a clinical cutoff in normolipidemic and dislipidemic subjects who showed no signs of CHD or diabetes mellitus at baseline (n =

3,938) in MESA. The established cutoff value was validated using individuals who participated in ARIC study Visit 4 (1996-1998). The study population was recruited from four U.S. communities, and the subjects who did not have CHD at the baseline (n=10,290) were followed for a maximum of 16 years' period. Proportional hazards regression analyses were used to investigate the association of incident CHD, defined as hospitalized myocardial infarction, fatal CHD, or cardiac procedure. Hazard ratios (HRs) of incident CHD were adjusted for Model 1: age, sex and race; and Model 2: Model 1 variables + ever smoker, BMI, hypertension, HDL-C, triglycerides (log-transformed), lipid-lowering medications, diabetes and hs-CRP (log-transformed). **Result:** In MESA, the 75th percentile value of sd LDL-C was analyzed as 48.4 mg/dL in the subjects with no CHD or diabetes mellitus, and this was rounded to 50.0 mg/dL as a clinical cutoff. Absolute risk and Cox proportional hazards regression analyses were used to investigate the association of incident CHD. Subjects were dichotomized by their baseline levels of sd LDL-C, and analyses were conducted using the group with sd LDL-C <50.0 mg/dL as reference. In Model 1, individuals in the higher sd LDL-C group showed approximately a 1.6-fold higher risk for incident CHD compared to the reference group (HR 1.55; 95% CI 1.39-1.73). In Model 2, risk for incident CHD was somewhat attenuated, but remained significant (HR 1.26; 95% CI 1.10-1.43). **Conclusion:** The cutoff value of sd LDL-C established as 50 mg/dL was validated for its clinical use in predicting the risk of CHD. This FDA-approved assay is promising for application in routine clinical practice as an IVD product.

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Lipoprotein Particle Stability in Serum for Nuclear Magnetic Resonance Analysis

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Background: Lipoprotein particles consist of cholesterol and other lipids characterized according to their composition, density, size and biological function. It is also known that independent of cholesterol content, lipoprotein particles, including their subclasses, can vary with respect to density, size and lipid content. Associations between the blood concentrations of these particles and increased coronary heart disease (CHD) risk, insulin resistance, diabetes mellitus and metabolic syndrome are well established. Consequently, lipoprotein-lipid profiling may better identify individuals with an increased risk of CHD. Methods for analyzing lipoprotein particles include gel electrophoresis, density gradient ultracentrifugation, ion mobility analysis, and nuclear magnetic resonance (NMR) spectroscopy. The stability of lipoproteins in biological samples is of importance in clinical settings. The purpose of this study was to assess the stability of lipoprotein particles in serum for analysis by NMR. **Methods:** Deidentified residual serum specimens sent to ARUP Laboratories for routine testing were used. Lipoprotein particle concentrations and sizes were measured using the AXINON® lipoFIT® test system incorporating the Bruker Ascend™ 600 Avance III HD NMR platform (numares AG, Regensburg, Germany) according to the AXINON test kit protocol. The University of Utah's Institutional Review Board approved this study. The lipoproteins evaluated were high-density lipoprotein particle number (HDL-p), large high-density lipoprotein particle number (LHDL-p), low-density lipoprotein particle number (LDL-p), small low-density lipoprotein particle number (SLDL-p), large very-low-density lipoprotein particle number (LVLDL-p), high-density lipoprotein particle size (HDL-s), low-density lipoprotein particle size (LDL-s) and very-low-density lipoprotein size (VLDL-s). Conditions included room temperature, refrigerated, frozen (-20 °C) and freeze/thaw cycles. **Results:** Summarized in the table below. **Conclusions:** Lipoprotein particles HDL-p, LHDL-p, LDL-p, SLDL-p, LVLDL-p, HDL-s, LDL-s and VLDL-s demonstrate reasonable stability in serum at room and refrigerated temperatures for analysis by NMR. However, untimely degradation is possible for LHDL-p, SLDL-p and especially, LVLDL-p for specimens stored frozen at -20 °C.

Lipoprotein Particle Stability

	Room Temperature	Refrigerated	Frozen (-20 °C)	Freeze/Thaw
HDL-p	min 48 hours	min 30 days	min 3 months	min 3 cycles
LHDL-p	min 48 hours	min 30 days	1 month	min 3 cycles
LDL-p	min 48 hours	min 30 days	min 3 months	min 3 cycles
SLDL-p	min 48 hours	min 30 days	2 months	min 3 cycles
LVLDL-p	min 48 hours	min 30 days	1 week	2 cycles
HDL-s	min 48 hours	min 30 days	min 3 months	min 3 cycles
LDL-s	min 48 hours	min 30 days	min 3 months	min 3 cycles
VLDL-s	min 48 hours	min 30 days	min 3 months	min 3 cycles

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Possible Concern of Erythrocytes with Reverse Cholesterol Transport

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Background: High-density lipoprotein (HDL) plays a main role in the reverse cholesterol transport (RCT) by taking up cholesterol (cholesterol efflux) from foam cells and carrying it to the liver. Recent studies have pointed out that cholesterol accumulation within the intima and expansion of the necrotic core are induced also by intraplaque hemorrhage and lysis of erythrocytes at the lesion site. One report showed blood cholesterol in mouse was possibly transported by erythrocytes at higher level when apolipoprotein A-I (apoA-I), the main apolipoprotein in HDL, was knocked down. Therefore, we investigated the participation of erythrocytes in RCT according to interacting with apoA-I and HDL. **Methods:** Cholesterol efflux capacity (CEC) was measured as we have previously described. THP-1 cells (human acute monocytic leukemia cell line) were stimulated by phorbol myristate acetate and differentiated into macrophages. Cells were then incubated with acetylated low-density lipoprotein for foam cell formation, ^{3}H-cholesterol as a tracer and LXR activator T0901317 for enhancing the expression of cholesterol transporters. To evaluate CEC, apoA-I or HDL as a conventional cholesterol accepter was incubated with foam cells for 4 hours in the presence or absence of various amounts of erythrocytes and the radioactivity in the medium, erythrocyte and cell lysate were measured. CEC was defined as the percentage of radioactivity distributed to the medium (apoA-I or HDL) and erythrocytes. Cholesterol transferred between apoA-I (or HDL) and erythrocytes was also determined by the incubation of ^{3}H-cholesterol acquired apoA-I (or HDL) and erythrocytes, obtained by CEC assays, with fresh erythrocytes and apoA-I (or HDL), respectively. **Results:** In the CEC assay including both apoA-I (or HDL) and various amounts of erythrocytes, the percentages of radioactivity in erythrocytes increased in a dose dependent manner, while those in medium including apoA-I or HDL decreased contrastively. Next, cholesterol transferred between apoA-I (or HDL) and erythrocytes was investigated using ^{3}H-cholesterol acquired apoA-I (or HDL) and erythrocytes obtained by CEC assay. The radioactivity of apoA-I and HDL in the medium decreased by approx. 81% and 51% after incubating with fresh erythrocytes, respectively. On the opposite direction, some ^{3}H-cholesterol acquired by erythrocyte transferred to fresh HDL but almost not to fresh apoA-I. Further, human serum albumin (HAS) tends to facilitate cholesterol efflux of erythrocytes without regard to existence of apoA-I or HDL; however, HAS did not affect cholesterol exchange between erythrocytes and apoA-I (or HDL). **Conclusions:** Erythrocytes might facilitate cholesterol efflux and increase CEC by receiving cholesterol which apoA-I and HDL take up from foam cells. Actually, cholesterol transport from ^{3}H-cholesterol acquired apoA-I and HDL to erythrocytes was observed in the condition without foam cells; however, its efficiency in apoA-I was higher than that in HDL. Cholesterol transport from ^{3}H-cholesterol acquired erythrocytes to HDL was also observed, but not to apoA-I. These could indicate that free apoA-I, a minor part of total apoA-I, plays as a predominant cholesterol accepter in cholesterol efflux according to a collaboration with erythrocytes. Consequently, erythrocyte may play an important role in RCT as a temporary cholesterol storeroom.

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Impact of fasting time on abnormal flagging rates when clinical decision limits are applied to total cholesterol, HDL-C, LDL-C (calculated), non-HDL-C, and triglycerides

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Background: Use of non-fasting lipids is gaining widespread acceptance for cardiovascular risk-stratification. However, many dyslipidemia guidelines have yet to provide recommendations for clinical cutoffs for non-fasting total cholesterol, HDL-C, LDL-C, non-HDL-C, and triglycerides and few studies have examined the impact of fasting time on the kurtosis and skewness of the distribution of lipid results and abnormal flagging rates. We hypothesized that variations in meal composition may broaden the distribution of lipid results and impact flagging rates in non-fasting specimens. Our objectives were to identify the proportion of patients presenting for lipid testing in a non-fasting state and to assess the impact of fasting time on: 1) the median concentration, kurtosis and skewness of results; 2) the abnormal flagging rate when clinical cutoffs are applied. **Methods:** A retrospective cross-sectional review of adult (≥18 years of age) lipid results (N=261,645 males and 280,651 females) obtained over a four-month period was performed. Abnormal flagging rates were based on the following desirable clinical cutoffs: total cholesterol<200 mg/dL; male HDL-C<40 mg/dL, female HDL-C<50 mg/dL; LDL-C<135 mg/dL; non-HDL-C<166 mg/dL; and triglycerides<150 mg/dL. Total cholesterol, HDL-C, and triglycerides were measured while LDL-C and non-HDL-C were calculated. Fasting duration was obtained by patient-report and results were partitioned by sex without age distinction. The flagging rate, calculated as the number of results exceeding the clinical cutoff/total number of results for that fasting time, was calculated for each hour of fasting. **Results:** Although mandatory fasting requirements for lipid measurements had been removed over two years prior to the study, only 35% of males and 37% of females presented for phlebotomy in a non-fasting state (0-11 hours for cholesterols, 0-7 hours for triglycerides). Consistent with previous studies, the median concentrations of total cholesterol, HDL-C, and LDL-C differed statistically but not clinically across fasting times (0-16 hours). No significant change was seen in non-HDL-C. Additionally, regardless of fasting status, the kurtosis and skewness of the distributions for these analytes remained consistent. However, the median concentration of triglycerides increased by ~20% and the distribution curve broadened between 0-7 hours as compared to 8-16 hours fasting. For all analytes, these changes were mirrored in their flagging rates. For males, between fasting and non-fasting, the average flagging rate changed from 27.8% to 28.1% for cholesterol; 21.4% to 24.1% for HDL-C; 18.1% to 15.9% for LDL-C; 18.3% to 19.3% for non-HDL-C; and 33.0% to 46.9% for triglycerides. Similarly, for females, between fasting and non-fasting, the average flagging rate changed from 38.1% to 37.9% for cholesterol; 26.9% to 28.2% for HDL-C, 20.2% to 17.9% for LDL-C, 17.8% to 17.9% for non-HDL-C, and 24.2% to 36.1% for triglycerides. In total, for our patient cohort, ~286,000 lipid test results, or ~13%, would be differentially flagged if all patients presented as non-fasting as compared to fasting. **Conclusion:** Dyslipidemia guidelines should consider providing adjusted clinical decision limits for non-fasting lipids, in particular LDL-C (if calculated) in order to improve clinical sensitivity. Distinct limits should be provided for fasting and non-fasting triglycerides to avoid substantial increases in abnormal flags in non-fasting specimens.

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High prevalence of diabetes mellitus type 2 among participants of Hipercol Ceará Program

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Background: The Familial Hypercholesterolemia (FH) is a genetic disease. It causes high LDL-cholesterol levels and high cardiovascular disease (CVD) risk. The Type 2 Diabetes Mellitus (T2DM) is an additional cardiovascular risk which can also occur in FH patients. Few studies on the prevalence of T2DM among FH patients have been reported. Data from international studies show a prevalence of T2DM among FH patients of less than 10%. In Brazil, the prevalence of T2DM is about 12.1% (SBD, 2016) and among FH cases, 14.7% (Santos et al., 2014). However, data about T2DM prevalence among FH patients is unknown. **Methods:** A cross-scetional study of patients evaluated in "Programa Genético de Rastreamento Ativo de Hipercolesterolemia Familiar do Ceará - HIPERCOL CEARÁ" at reference service from 2013 to 2017. The patients evaluated had a level of LDL- cholesterol level above 210 mg/dL, besides that clinical characteristics, comorbidities, lifestyle and family history to early CVD. A molecular study was performed for gene mutation analysis (LDL receptor, ApoB protein and inhibitor protein PSK9). The first degree relatives (FDR) of genetic confirmed cases were also evaluated. **Results:** A total of 122 participants were evaluated; 34 had LDL receptor gene mutation and 24 was previously diagnosed for T2DM (mean age=57.8 (±12.4) years; female gender= 83.3%). About origin, 18 (75%) lived in the capital, 4 were from interior state and 2 (8.3%) lived in Fortaleza Metropolitan Region. The T2DM prevalence among the index cases was 87.5% and 12.5% in their relatives. About the suspect cases, the T2DM prevalence was 19.7%. When considering only the confirmed cases, the T2DM prevalence was 20.8%. **Conclusion:** It is observed a high prevalence of T2DM in the evaluated population being superior to that described in another Brazilian population. A larger study is needed, but these data may suggest the need for early detection of T2DM among FH patients.

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Lipid profile, Oxidative Stress & Anti-Oxidative Status of Paraoxonase Enzyme: Comparative Approach in Nepalese Obese & Non-obese People

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Background
Obesity is a condition of excessive body fat accumulation adversely affecting health. It is associated with lipoprotein peroxidation leading to atherogenesis. Paraoxonase enzyme associated with high–density lipoprotein (HDL-PON) exerts a protective effect against oxidative damage of circulating cells and lipoproteins.
Objective
We investigated the relationship between lipid profile, total peroxide (measure of oxidative stress) & serum paraoxonase (HDL associated) aryl esterase activity (measure of antioxidative status) in non–obese and obese Nepalese people.
Methods
Anthropometric variables including BMI, serum lipids, total peroxide and PON ARA were measured in, and compared between, consenting age-matched (33.75±0.82 yrs.) non-obese (n=105) and obese (n=105) subjects. The data are as mean±SE.
Results
The concentrations of total cholesterol (obese 188.63±6.71 Vs non-obese 147.56±3.24, mg/dl, p<0.001), triglycerides (obese 189.12±9.96 Vs non-obese 134.53±6.69, mg/dl, p<0.001) and LDL cholesterol (obese 109.97±6.06 Vs non-obese 75.75±2.90, mg/dl, p<0.001) were significantly high in obese subjects. Serum PON ARA (obese 124.23±9 Vs non-obese 184.10±13.80, µmol/min/ml, p<0.01) and HDL (obese 40.82±0.66 Vs non-obese 44.88±1.19, mg/dl, p<0.01) were significantly lower and total peroxide level (obese 19.29±0.54 Vs non-obese 12.77±0.25, µmol H_2O_2/liter, p<0.001) was higher in obese than in non-obese. Negative correlation found between PON ARA and total peroxide level confirms the relation between paraoxonase activity and lipoprotein lipid-peroxidation.
Conclusion
The findings suggest an increase in the level of bad cholesterol along with reduced level of good cholesterol, increase level of oxidative stress in obesity, which is associated with a decrease in HDL-PON activity and increased risk of cardiovascular diseases among people with obesity in least developed country, Nepal. This study would suggest guiding the development of policies to reduce the burgeoning issue of obesity & thus the burden of metabolic syndrome & non-communicable diseases in least developed country in Asia, like Nepal.

B-158

Performance Evaluation of an Automated Assay for the Measurement of LPL and HL Activity

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Background: Lipoprotein lipase (LPL) hydrolyzes triglycerides (TGs) into chylomicrons and VLDL particles during lipoprotein metabolism. Similarly, hepatic lipase (HL) is synthesized by hepatocytes and hydrolyzes TGs and phospholipids

in chylomicron remnants, intermediate density lipoproteins and HDLs. LPL deficiency leads to hypertriglyceridemia with accumulation of chylomicrons. HL deficiency leads to hypercholesterolemia, hypertriglyceridemia and accumulation of β-VLDLs, chylomicron remnants, IDLs, TG-rich LDLs and HDLs. The conventional method for measuring LPL and HL activity uses ^{3}H- or ^{14}C-labeled trioleoyl glycerol and is not suitable for routine clinical measurement. A novel assay has been developed which is applicable to automated clinical analyzers. Here, we evaluated the performance of the new LPL and HL activity assay method in human post-heparin EDTA plasma (PHP) using the cobas c501 autoanalyzer. **Methods:** LPL and HL activities were measured colorimetrically using two different channels on the cobas c501 autoanalyzer (Roche Diagnostics). The first channel contained apoCII, a cofactor required for LPL activity, and measured combined LPL and HL activities. The second channel lacked apoCII, measuring only HL activity. LPL activity was calculated from the difference between the two channels. The performance of the two channels as well as the calculated LPL activity were evaluated and validated using PHP through several experiments, including precision, linearity (2 samples), recovery (2 samples spiked to 3 different levels), sensitivity, reference interval (20 subjects) and stability (3 single donors). The within-run precision (WRP) and between-run precision (BRP) were evaluated using three in-house plasma controls with three different concentrations of LPL+HL and HL activities. **Results:** For precision of both activity channels, coefficients of variation (CV) for all controls ranged from 0.9 – 4.5% for WRP, and 2.8 – 7.4% for BRP. For linearity, acceptable results ranged from 82.9 – 119.5% of targets with up to 16-fold dilution for both activity channels. For recovery, post-heparin plasma spiked into pre-heparin plasma demonstrated acceptable recovery of LPL and HL activities ranging from 100.0 – 117.1% of targets. The sensitivities for LPL+HL, HL and LPL activities in PHP were identified at 5, 17 and 11 U/L, respectively, with precision of ≤ 20%CV. For the reference interval, LPL and HL activities were measured in 20 PHP samples from normal healthy volunteers. These results aligned with the range obtained by the manufacturer with values of 42 – 209 U/L and 198 – 859 U/L for LPL and HL activity, respectively. Accuracy was also verified using commercially available control samples and ranged from 90.0 – 108.6% of target. For short-term stability in post-heparin plasma, LPL+HL and HL activities were stable at 2 – 8 °C for up to 3 days, up to 1 day at room temperature, and stable for 3 additional freeze-thaw cycles. **Conclusion:** The utility of the LPL/HTGL Activity was demonstrated with sufficient analytical performance. Overall, this assay on an automated platform is ideal for measuring LPL and HL activities in clinical trials.

B-159

Dapagliflozin decreases sd LDL-C and increases HDL2-C in patients with type 2 diabetes comparison with sitagliptin

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BACKGROUND: Several recent studies have reported that sodium-glucose co-transporter-2 (SGLT-2) inhibitors increase both low-density lipoprotein (LDL) and high-density lipoprotein (HDL)-cholesterol (C). In this study, we determined the effect of SGLT-2 inhibitors on LDL and HDL-C subspecies. **METHODS**: Single center, open-label, randomized, prospective design was employed. 80 patients with type 2 diabetes taking prescribed oral agents were allocated to receive SGLT-2 inhibitors, dapagliflozin (n=40) or dipeptidyl peptidase-4 inhibitor, sitagliptin (n=40) as add-on treatment. Fasting blood samples were collected before and 12 weeks after this intervention. Small, dense (sd) LDL-C, large buoyant (lb) LDL-C, HDL2-C, and HDL3-C were measured using our established homogeneous assays. **RESULTS**: Dapagliflozin and sitagliptin comparably decreased HbA1c (0.75 and 0.63%, respectively). Dapagliflozin significantly decreased body weight, systolic blood pressure, plasma triglycerides and liver transaminases, and increased adiponectin; sitagliptin did not affect these measurements. For the patients with dapagliflozin treatment, no significant change was observed in their LDL-C and apolipoprotein (apo) B levels, whilst their HDL-C and apo AI were increased. Interestingly, however, we found that sd LDL-C decreased by 20% and lb LDL-C increased by 18%. The level of lb LDL-C was remarkably elevated (53%) in individuals (n=20) with elevated LDL-C by dapagliflozin, whilst sd LDL-C remained suppressed (20%). Dapagliflozin increased HDL2-C by 18% without affecting HDL3-C. Sitagliptin did not alter plasma lipids or lipoprotein subspecies. **CONCLUSIONS**: Dapagliflozin suppressed potent atherogenic sd LDL-C and increased HDL2-C, a favorable cardiometabolic marker. Although LDL-C levels are elevated by treatment with dapagliflozin, this was due to increased concentrations of the less atherogenic lb LDL-C. However, these findings were not observed after treatment with sitagliptin.

B-160

Direct Lipoprotein Measurements and Cardiovascular Disease Risk

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Objectives: Cardiovascular disease (CVD) is a major cause of death and disability in the United States. The standard American Heart Association (AHA) model for CVD risk assessment includes age, gender, systolic blood pressure, use of blood pressure medication, history of diabetes, history of current smoking, total cholesterol (TC), and high density lipoprotein cholesterol (HDL-C). Elevated serum levels of low density lipoprotein cholesterol (LDL-C), small dense LDL-C (sdLDL-C), remnant lipoprotein cholesterol (RLP-C), and lipoprotein (a) or Lp(a) and low HDL-C have all been associated with an increased risk of CVD, including coronary heart disease, stroke, peripheral vascular disease, coronary revascularization, and CVD mortality. Our objectives were to assess direct measurements of these lipoproteins as compared to standard risk factors in the prospective Framingham Offspring Study. **Methods:** Stored frozen plasma samples (-80 degrees C) obtained after an overnight fast from male and female participants free of all CVD at cycle 6 of the Framingham Offspring Study were used (n=3,147, mean age 58 years). A total of 677 subjects or 21.5% developed a CVD endpoint over a 16 year period of follow-up. TC, HDL-C, direct LDL-C, sdLDL-C, RLP-C, Lp(a), and high sensitivity C reactive protein (hsCRP) were measured by standardized automated analysis. All assays had within and between run coefficients of variation of < 5%. Estimated LDL-C was calculated as: total cholesterol - HDL-C - TG/5 provided subjects had fasting triglyceride values < 400 mg/dL. Statistical analysis included logistic regression, multivariate modeling, and net reclassification. **Results**: For CVD risk on univariate analysis significant factors with p values in parentheses in order of significance were: age (8.1×10^{-41}), hypertension (3.2×10^{-23}), HDL-C (4.2×10^{-16}), sdLDL-C (4.2×10^{-14}), hypertension treatment (1.5×10^{-14}), gender (1.7×10^{-10}), diabetes (5.1×10^{-9}), direct LDL-C (8.2×10^{-9}), body mass index (9.2×10^{-7}), calculated LDL-C (6.2×10^{-6}), RLP-C (8.0×10^{-4}) cholesterol medication (1.8×10^{-4}), total cholesterol (0.00081) smoking (0.0024), hsCRP (0.005), and Lp(a) (0.024). On multivariate analysis sdLDL-C, direct LDL-C, hsCRP, and Lp(a) were all still significant using the model including all standard risk factors. All four parameters significantly improved the model C statistic and net risk reclassification. **Conclusions:** Our data indicate that: 1) HDL-C and sdLDL-C are the most significant lipoprotein predictors of CVD; 2) calculated LDL-C underestimates direct LDL-C levels, 3) direct LDL-C is significantly better than calculated LDL-C in CVD risk prediction; 4) HDL-C, sdLDL-C, direct LDL-C, RLP-C, and Lp(a) are all significant lipoprotein particles contributing to CVD risk, and 5) with all standard risk factors in the model sdLDL-C, direct LDL-C, Lp(a), and hsCRP all add significant information above and beyond the standard model in predicting CVD risk prospectively over about a 16 year period in the Framingham Offspring Study. We conclude that these parameters should be measured in all patients with CVD, diabetes, or those with an AHA calculated 10 year CVD risk of ≥ 7.5% (i.e patients targeted by AHA for diet and statin treatment).

B-161

Fasting versus Non-Fasting Lipid Panels

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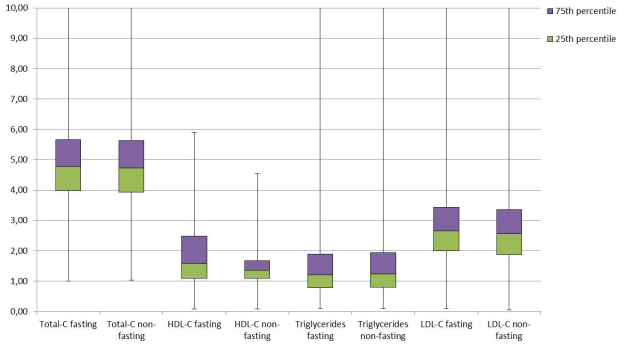
Background: In 2016 a European guideline was introduced by Nordestgaard *et al.*, advising for patients not to fast before having their lipid levels tested[1]. Non-fasting lipid testing is more convenient for the patient, could reduce laboratory costs by spreading tests on a workday, but also may better represent a typical lipid load throughout the day, as was explained in the AACCs' January/February *Clinical Laboratory News*. The aim of our study was to retrospectively measure whether the introduction of a non-fasting policy for lipid testing in our hospital changed the test results, including the LDL-C (low-density lipoprotein-cholesterol) calculated with the Friedewald formula. **Methods:** Of each lipid test (Total Cholesterol, HDL-C, Triglycerides, and LDL-C) we compared at least 10,000 results before the introduction of the non-fasting policy for lipid testing to at least 10,000 results after. Differences of the mean, median and standard deviations were calculated with EP Evaluator. **Results:** The fasting versus non-fasting lipid panels showed a very good correlation. Differences in the mean and median were very small (0,009-0,111mmol/L and 0,00-0,09mmol/L, respectively) and not significant. Box-plots are presented in the figure. Results are in mmol/L. **Con-**

clusion: In concordance with previous studies, the non-fasting lipid panels in our hospital do not significantly differ from fasting lipid panels. Because of the advantages of non-fasting lipid testing, this is the method of choice. **References:** Nordestgaard *et al.* 2016. Clin Chem 62:7, 930-946 and CLN Stat. Feb.1.2018.



Wednesday, August 1, 2018

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

Management

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National Survey on Sigma Analysis of Appropriateness of Laboratory Reports in 2017 in China

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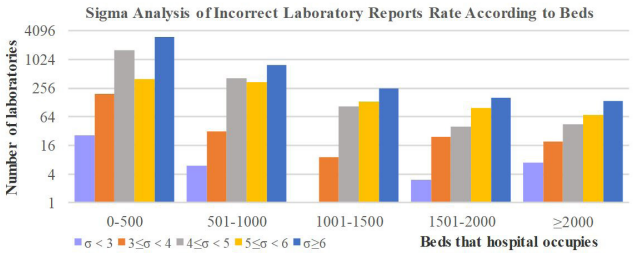
Background: Appropriateness of laboratory reports is a part of post-examinational process. Three quality indicators (QIs) about it have been developed in China, including incorrect laboratory reports rate, critical values notification rate and timely critical values notification rate. This study aimed at analyzing sigma level of these three QIs. **Methods:** 9039 clinical laboratories from 31 provinces enrolled in external quality assessment programs for QIs of clinical laboratories in 2017. General information and related data were asked to be submitted via net platform established by National Center for Clinical Laboratories (NCCL). The results were evaluated with sigma scales (σ) and percentage. Mann-Whitney Test and Kruskal-Wallis Test were used to perform the group comparison. **Results:** 7633(84.5%), 7169 (79.3%) and 6974(77.2%) laboratories submitted data of three QIs, respectively. Among laboratories provided data about incorrect laboratory reports rate, 6319 laboratories reported beds their hospital occupied. As caculated, most labs had three QIs at σ≥6, even more than 6000 labs had critical values notification rate at world class level. For incorrect laboratory reports rate, even though a large number of laboratories got the lowest defects per million opportunity, there were still a certain number of laboratories got unacceptable sigma level for each group. **Conclusions:** Although overall sigma level of these three QIs in 2017 in China was satisfied, but there were some problems if data were grouped by different methods like beds that hospital occupied. Therefore, there is still space for labs to improve the quality of testing reports.



B-163

Strategies for the implementation of the ISO 15189 standard in clinical laboratories in Mexico

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The ISO 15189 accreditation is a specific international standard for clinical laboratories (CL), which allows demonstrating quality and technical competence. In Mexico this accreditation is voluntary, placing this country in second place in Latin America and the Caribbean, in terms of the number of laboratories accredited by this standard. However, these laboratories only represent 1.97% of the total LC in Mexico, so it is convenient to analyze the strategies used by the currently accredited laboratories, with the intention of disposing elements that facilitate the implementation and accreditation to other laboratories. Mexico has a mandatory standard for the operation of CL that does not require accreditation; however, ISO 15189 accreditation is available as a voluntary standard since 2005, by the national accreditation body called the Mexican Accreditation Entity. The objective of this research was to establish strategies for the implementation and accreditation of Quality Management Systems (QMS) under the ISO 15189 standard in CL in Mexico, identifying the main problems detected in the implementation, the strategies used and alternative proposals to increase the number of accredited laboratories.For the methodology, it was considered a quantitative, descriptive, cross-sectional investigation, using the survey as a technique and the questionnaire as an instrument, which was previously validated according to the criteria of Rivas (2004). A probabilistic sampling without replacement was used, with a confidence level of 90%, for a finite population of accredited laboratories, involving a representative sample of 44 CL and 3 accredited blood banks (BB). The sample size was determined by looking for it to be statistically representative of the universe that was constituted of 106 CL and 6 BB accredited at the time of the investigation.The information was tabulated using the statistical program STATA version 14, elaborating contingency tables. To analyze the relationship between variables, the Pearson Chi^2 test was used. To estimate the association between variables we used Cramer's V, Kendall's tau-b and Goobman's and Kruskalll's Gamma.As a result of the analysis of the information, it was identified that: 1. The knowledge and commitment of the personnel, 2. The previous experience in QMS and 3. The counting in the country with free technical documents to meet technical requirements considered critical such as validation of methods, traceability and measurement uncertainty, are key elements for CL accreditation. The results obtained also allowed to verify that the accreditation is accessible to any Mexican laboratory, regardless of its size or complexity, being convenient to train the personnel in QMS.In conclusion: the commitment of the personnel, the training of the human resource, the availability and use of free technical guides facilitate the ISO 15189 accreditation.

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Summary and Analysis of Blood Culture Contamination Rate in Clinical Laboratories in China from 2015 to 2017

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Background: Blood culture is a long-term test carried out by clinical microbiology laboratory, the positive results of which have great significance. Blood culture contamination rate is one of the 15 quality indicators (QI) of examination procedures published by National Health and Family Planning Commission. In the research, we collect and process the data obtained from clinical laboratories around the country in 2015, 2016 and 2017 to observe the status of blood culture contamination rate. **Methods:** By using the software developed by National Center for Clinical Laboratories (NCCL), we can get the data of blood culture contamination rate from 2015 to 2017. The proportion of clinical laboratories is classified according to the different level of blood culture contamination rate and we also look into the contamination rate by hospitals with different beds to identify the corresponding status in hospitals of different rank. Microsoft office Excel 2007 is applied to finish the calculation. **Results:** There are 3065, 4487, and 4826 laboratories taking part in this survey from 2015 to 2017, as illustrated in the table below. In the left column we can figure out that blood culture contamination rate is mainly distributed in 0 to 0.25, which are 48.32%, 48.21% and 47.70%, separately, while the ratios in other rows are small and decentralized, indicating a good control in blood culture contamination rate. Hospitals with more than 2000 beds get the lowest blood culture contamination rate on the basis of mean value, compared with hospitals possessing a smaller number of beds. **Conclusions:** To sum up, the quality control of blood culture contamination rate in Chinese clinical laboratories is relatively good, but there are still some cases having an unsatisfactory quality control in contamination rate. Therefore, hospitals and clinical laboratories should work together to find the causes behind and make their best effort to achieve a better practice.

Year	Basic situation			Situation of hospitals with different beds				
	Classification (blood Culture contamination rate) (%)	Laboratories Number	Percentage (%)	Classification (number of beds)	mean	P25	P50	P75
2015	0-0.25	1481	48.32	0-500	2.52	0	0	2.31
	0.25-1	350	11.42	501-1000	2.29	0	1.09	2.86
	1-1.75	347	11.32	1001-1500	2.79	0.38	1.21	2.86
	1.75-2.5	207	6.75	1501-2000	2.00	0.54	1.25	2.93
	2.5-3.25	163	5.32	>2000	1.69	0.43	1.15	2.30
	3.25-10	517	16.87					
2016	0-0.25	2163	48.21	0-500	3.25	0	0	2.50
	0.25-1	540	12.03	501-1000	2.20	0	0.95	2.57
	1-1.75	484	10.79	1001-1500	2.60	0.23	1.06	2.45
	1.75-2.5	360	8.02	1501-2000	1.80	0.34	1.08	2.08
	2.5-3.25	243	5.41	>2000	1.75	0.41	1.25	2.42
	3.25-10	697	15.53					
2017	0-0.25	2302	47.70	0-500	2.72	0	0	2.00
	0.25-1	604	12.52	501-1000	2.08	0	0.78	2.17
	1-1.75	546	11.31	1001-1500	2.28	0.26	1.00	2.11
	1.75-2.5	310	6.42	1501-2000	2.62	0.34	0.96	1.79
	2.5-3.25	236	4.89	>2000	1.37	0.24	0.94	2.12
	3.25-10	828	17.16					

B-165

Long term variation in the quality control measurements of three contemporary hemoglobin A1c assays easily demonstrates effects of biased testing: a new tool in the laboratorian's QC armamentarium

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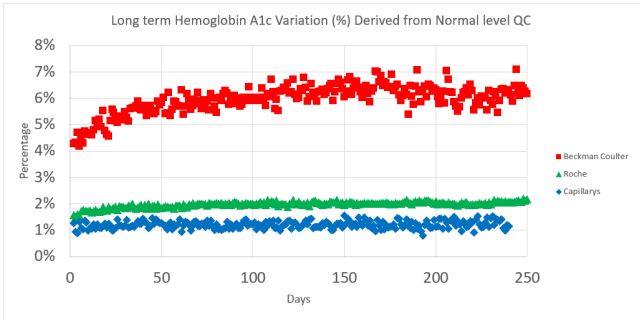
Introduction: We determine the long term (LT) variation of quality control (QC) specimens and apply this variation to the interpretation of the analysis of patient specimens. Repeated QC measurements can be considered analogous to the repeated measurement of a single patient specimen over months or years. For hemoglobin A1c (HbA1c) interpretations, a measurement of a prior specimen is compared to a measurement of a new specimen. In our model, the variation of all possible QC pairs is calculated; QC results are grouped with subsequent QC results as long as the time interval between the two QCs is the same. The standard deviation of the differences (SDD) of the groups of QC pairs provides an average variation for each time interval. Graphs of the LT QC SDD easily illustrate the effect of reagent lot variation or closely spaced biased analytical runs. **Materials and Methods:** QC data were obtained from a Quebec laboratory for Beckman Coulter Synchron DxC®immunoassay and the Capillarys 2 Flex Piercing® (C2FP), and from a New Hampshire hospital, the Roche Tinia Quant Gen III, Cobas 8000, c502. and Cobas 6000, c501. We generated graphs of the LT QC SDD variation for the three methods for the available QC levels. **Results:** The Figure compares the LT QC SDD of the three assay's normal level A1c analysis. The traditional QC chart of the Beckman data (not shown) demonstrates sporadic intermediate term biases. **Discussion:** The LT graphs demonstrate the superiority of the Roche and Capillarys assays (we maintain that HbA1c imprecision should not exceed 3%). With reference to the Beckman assay, even QC results separated by 10 to 20 days begin to demonstrate high variation compared to prior results. We recommend that suppliers of quality control product begin to provide such long term variation analyses for all of their analytes.



B-166

Vitamin D request in Primary Care. Is it Really Pandemic?

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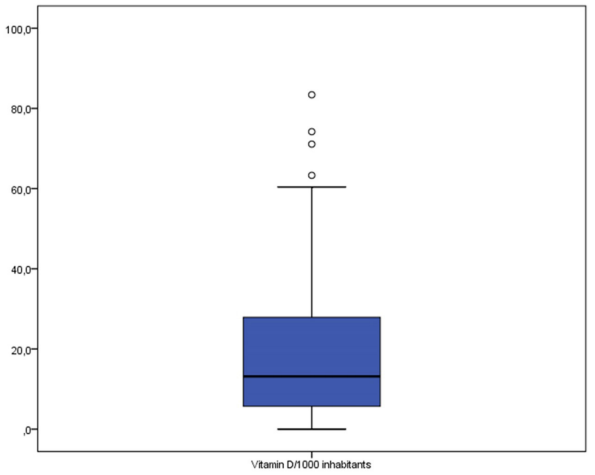
Background: The request of 25[OH]D by general practitioners (GPs) in Spain doubled in a recent period of two years. Such over-screening might result in unnecessary prescriptions of supplemental vitamin D. Our goal was to study the current request of 25[OH]D in Primary Care to evaluate its evolution over-time, and a potential over-request correction. **Methods:** Clinical laboratories from the Redconlab working group were invited to report the number of 25-hydroxyvitamin D (25(OH)D) tests requested by GPs during the year 2016 and number of individuals covered by their health departmentes. The number of 25(OH)D requested per 1000 inhabitants and the index of variability (90th percentile/10th percentile) were calculated. Economic cost taking into account prices reported by the participants were also calculated **Results:** Seventy-seven laboratories participated corresponding to 19222006 inhabitants (41.3% of the Spanish population). The number of 25(OH)D requested was 426406, that corresponded to an expense of 2166142.5 euros. That would mean 5244897.1 euros if extrapolated to the entire Spanish population. 13.2 tests per 1000 inhabitants were requested (Figure), with a variability index of 61. **Conclusion:** 25(OH)D request was even higher than that observed in the two previous Redconlab editions, as was the variability index, suggesting increasing differences between geographical areas. The total expenses were significant for a test with such specific conditions for its request. From the laboratory, and in consensus with GPs, it is necessary to design interventions for an optimal request of a test that is not recommended for routine screening.



B-167

Factors Affecting The Interests Of Medical Students To The Study Of Laboratory Medicine/Pathology At The University Of Calabar Medical School

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Good medical practice and management of patients begins with making the right diagnosis. This leads to the right treatment of a disease entity. Laboratory medicine/pathology has to do with the study of diseases and their diagnoses. Adequate exposure of medical students to laboratory medicine will enhance their ability to become good clinicians. The interest of students to a particular subject may be influenced by different external and personal factors. This study aimed at investigating the factors affecting the interest of medical students to the study of laboratory medicine at the University of Calabar medical school. A total of 139 students involving two sets of medical students in their fourth year of study were randomly selected for this study. A 17-item questionnaire was used for data collection The study showed that gender, socio-economic class, number of hours spent on reading, good academic background, a positive attitude, and good subject perception enhanced interest in the study of laboratory medicine; while poor learning environment, low level of exposure, poor understanding of the subject had negative effects on medical students.

KEYWORDS: Medicine, Calabar, students, pathology, university, interest

B-168

Establishment and implementation of an improvement plan using lean six sigma to minimize variation in the ordered lab request

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Background:
Laboratory total testing process (TTP) encompasses internal and external laboratory activities that require interaction between laboratory personal and other specialists. The TTP can be divided into five phases; pre pre-analytic, pre analytic, analytic, post-analytic and post post-analytic phases. Test ordering is a part of the pre pre-analytical lab phase which is high error prone. Many of the tests ordered are unnecessary where excess tests ordering represent as much as 25–40% of all tests. Owing to the scarce data worldwide concerning the application of Six Sigma in pre pre-analytical lab phase, and the fact that it was not applied on the tests ordering process before, it was noteworthy to work on.
Aim of the work:
The present study targeted the pre pre-analytical lab phase aiming at establishing and implementing an improvement plan using Lean Six Sigma methodology (DMAIC) to minimize variation in the ordered lab requests.
Method:
The Define phase of the current improvement project, started by selecting the department with the highest work load. The Hepatology department represented 18.83% of the total number of lab tests ordered at the MRI hospital. Stakeholder analysis was the first tool performed in order to better understand the identified problem. The Project Charter was the last step in the Define phase. It included the business case, the current and desired Sigma levels, and the estimated savings achieved from the current project.
The Measure phase started by making the Flow Chart of the lab request tests ordering process Brainstorming sessions were then held in order to identify the possible root causes for the problem of the variation in ordering lab requests.
Results:
The Analyze phase started by prioritizing the root causes of the problem using a prioritization matrix. Pareto chart was used to identify the vital few causes that are responsible for nearly 80% of the problem of variation in lab tests request ordering process. The identified four main causes are; the ordering of unindicated tests as (ALT/AST ordered together), lack of awareness of evidence based medicine (EBM) basics, ordering certain tests in frequency that is not complying with biological variation (BV) as (Urea and Creatinine), and awareness of BV concept. The Sigma level for ALT/AST ordering process was calculated to be 0.66 and that of Urea and Creatinine was 1.23.
The Improve phase aimed mainly at creating solutions for the root causes selected during the Analyze phase. The Sigma level of ALT/AST tests ordering process became 1.2 Sigma which represents 45% improvement. The combined ordering of ALT and AST after improvement was significantly lower when compared to ordering of ALT and AST before improvement (P = 0.035). The Sigma level of Urea and Creatinine became 2.16 Sigma which represents 43.1% improvement.
Conclusion:
- Educational sessions and conjoint meetings between physicians and laboratorians are essential for any possible improvement initiatives.
- A simple change in the lab request form could be an important source of improvement and a cost reduction tool in the pre pre-analytical lab phase.

B-169

Demand management and optimisation, using precious laboratory resources wisely

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In 2017 we performed 19.5 million tests across the department of Pathology and Laboratory Medicine, serving the needs of a large tertiary referral hospital specialising in transplantation and oncology. With a 7% year on year increase in testing volume (average for the last 5 years, and 11.2% from 2016 to 2017) with fixed budgets, it was timely to review testing demand and to design strategies to maximise our precious resources; to ensure that we used them wisely. We have a duty to challenge and ensure that the right tests are performed on the right patients at the right time to drive optimal outcomes. Our objective was to explore the impact (financial and work volume) of time restricting tests that add no clinical value. To that end, a literature review was undertaken to explore international experience with lock-out frequencies (minimal re-test intervals). Using this guidance we engaged with clinical colleagues and agreed to pilot an approach to demand management with common tests that were high volume, high cost or perceived to be over used within our institution. We identified an initial list of common tests, over-subscribed in our institution, to lock-out that the literature supports added no clinical value. This locked-out group of tests reduced by an overall average of 6.6% versus the previous year, and saved 2.05 million Saudi Arabian Riyal (SAR) during a 6 month period. Given that we were growing at +7% year over year (preceding 5 year average), this means that the testing volume reduced by approx. 13% in real terms for all tests in this pilot study. There was a demonstrable reduction in work volume with associated cost avoidance; but more importantly this reduced the number of blood samples being collected from patients that added no clinical value. Due to the success of the pilot we continued to develop and add more tests to the lock-out list by implementing minimal re-test interval rules in the computer system to manage demand and ensure that we use our precious resources wisely.

B-170

Biological Variation of Serum Glycated Albumin in Chinese Healthy Population

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Background: European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working groups challenged existing biological variation (BV) and established a checklist for critical appraisal of studies of BV to establish a reliable and valid BV database. The BA data of glycated albumin (GA) was from a literature and needed to improve. This study aimed to estimate BV, individual index(II), reference change value(RCV) of GA in Chinese healthy population under the standard operating protocol.
Methods: Nineteen healthy subjects (males 9, females 10, aged between 21-35 years old) in Chengdu,a city in southwest China were enrolled in this study. Blood samples were obtained from the same phlebotomist every two weeks at the same time, for 3 months with a total of 5 times. Serum were separated and stored at -80 °C. All samples were thawed at room temperature uniformly. GA was measured in Roche Modular-P800 automatic biochemical analyzer in duplicate in the same analytical batch. The data were analyzed using CV-ANOVA test. BV, II and RCV of GA were calculated ultimately.
Results: For all subjects, males and females, the analytical variation (CV_A) of GA were 1.62%, 1.73%, 1.66%. The within-subject biological variation(CV_I) were 1.25%, 1.22%, 1.38%. The between-subject biological variation(CV_G) were 6.44%, 5.04%, 3.86%. II were 0.19, 0.24, 0.36 and RCV were 5.68%, 5.87%, 5.97%, respectively.
Conclusion: BV of GA estimated under the standard operating protocol were obviously lower than the data reported in the online database as expected (CV_I:1.25% VS. 5.2% and CV_G: 6.44% VS. 10.3%). The low II indicated the diagnostic effectiveness of population-based reference interval of GA was limited.

B-171

National Survey on Internal Quality Control Practice for Biochemistry Tests of Cerebrospinal Fluid in Clinical Laboratories in China

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Background: The biochemistry tests of cerebrospinal fluid (CSF) is important for the diagnosis of central nervous system disorders. In this survey, the internal quality control (IQC) data of clinical laboratories in China is analysed so as to have a knowledge of the current situation of impression level of measurement procedures in Chinese clinical laboratories in 2017. **Methods**:The data and clinical laboratories information including albumin(Alb), total protein(TP) , chloridion(Cl), glucose(Glu), lactic dehydrogenase(LDH), lactic acid(Lac), IgA, IgG and IgM are obtained via the external quality control (EQA) software developed by National Center for Clinical Laboratories(NCCL) in 2017. By comparing cumulative CVs with 1/3TEa or1/4TEa which is the quality standards published by NCCL for Chinese clinical laboratories and doing some mathematical calculation, we can get the proportion of laboratories meeting quality requirements. Computation is finished with the use of Microsoft office Excel 2007. **Results**:As shown in the following table, there are 85, 277, 311, 323, 134, 30, 52, 29 and 67 laboratories submit their data on the corresponding CSF biochemistry test items in 2017. Most biochemistry tests of cerebrospinal fluid carried out by clinical laboratories get a good IQC evaluation judging from the table. We can find that no matter which quality requirement is applied, 1/3TEa or 1/4TEa, the LDH always have the best IQC practice in clinical laboratories among these tests. However, the results of Alb and TP are not satisfactory with the proportion lower than 50%. What is noteworthy is that for IgA, IgG and IgM, the number of participant clinical laboratories is very small, from 29 to 52, thus, the result is unrepresentative. **Conclusion:** The bulk of biochemistry tests of CSF have a relatively good IQC practice, but for the specific items such as Alb and TP with unsatisfactory impression level, clinical laboratory should do maximum possible efforts to make suitable IQC plans to improve them.

Analytes	Number of participant laboratories	Cumulative CVs		Proportion of laboratories meeting quality requirements	
		Median of CVs (%)	IQR of CVs (%)	1/3 TEa	1/4 TEa
Alb	85	3.42	3.00	42.35%(36/85)	29.41%(25/85)
TP	277	4.04	3.85	34.66%(96/277)	20.22%(56/277)
Cl	311	1.43	0.86	58.52%(182/311)	30.55%(95/311)
Glu	323	2.00	1.37	75.23%(243/323)	59.13%(191/323)
LDH	134	2.50	1.74	86.57%(116/134)	77.61%(104/134)
IgA	30	4.79	3.51	73.33%(22/30)	60.00%(18/30)
IgG	52	4.19	2.06	82.69%(43/52)	73.08%(38/52)
IgM	29	4.80	2.23	72.41%(21/29)	68.97%(20/29)
Lac	67	2.69	2.23	80.60%(54/67)	76.12%(51/67)

B-172

Evaluation of insulin measurements performance through external quality assessment surveys from 2015 to 2017 in China

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Background: Insulin is an important anabolic hormone which is tested to assist in differentiating type 1 or type 2 diabetes and identifying insulin resistance. Due to its important role in diagnosing and monitoring diabetics, the quality assurance of insulin measurement is of great significance for clinical laboratories. **Methods:** Results from External Quality Assessment (EQA) program for insulin is carried out by the National Center for Clinical Laboratories(NCCL) in china from 2015 to 2017. Five levels of samples of which the homogeneity and stability were assessed referring to ISO 13528 were shipped to participant laboratories biannually.Data from participants were analyzed in different instrument group through robust statistics based on ISO 13528. The target value was determined by the robust median in each group. The performance criteria was target value±25% and comparability criteria was intergroup CV<8.33%(1/3 total error). intergroup CV and all-method CV were used to evaluate insulin measurement performance. **Results:** The number of laboratories increased gradually from 1091 to 1341 while pass rate maintained above 90% from 2015 to 2017. The all-method CV was higher in 2016 (A:20.49%-22.31%)than other years(A:12.58%-16.56%)in the low level samples.(Fig.1) The intergroup CV of low and high level were 13.11%-19.45%(D)and 10.67%-23.27%(E) respectively which were all above 8.33%. (Fig.1) **Conclusion:** The insulin measurement has been well-performed from 2015 to 2017, despite all-method CV of low level was higher in 2016 than other years. The results of different instrument group were not comparable which are probably caused by matrix effects. Therefore, it is essential to adopt commutable samples and improve standardization of insulin measurement for further efforts.

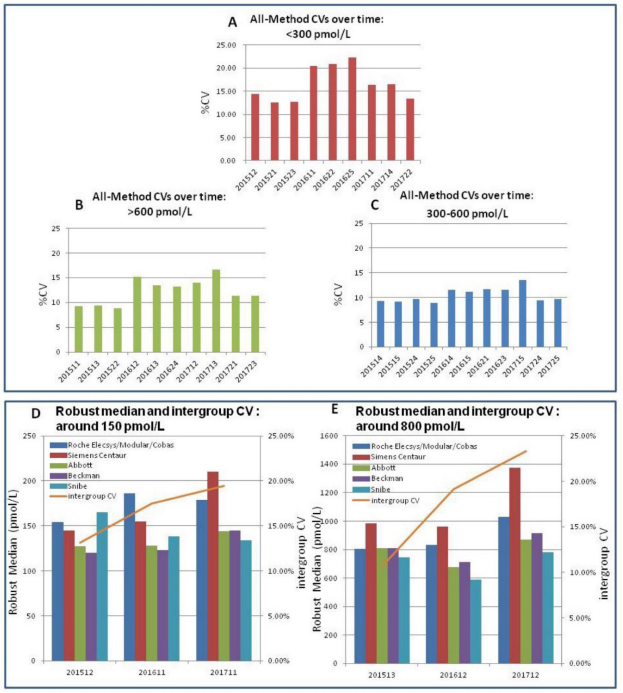

Figure 1: A,B and C show robust CV for all-method from 2015-2017 with assigned insulin values of (A)<300 pmol/L,(B)300-600 pmol/l,(C)>600 pmol/L; D and E display robust median of each group and intergroup CV from 2015-2017 with 2 level :(D) around 150 pmol/L,(E) around 800 pmol/L, the X-axis displays the material lot in each level through 3 years.

B-173

Imprecision investigation and analysis of internal quality control of five hepatic function tests in clinical laboratories of China

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Background: This study aimed to investigate the imprecision of the five most important analytes in diagnosing and monitoring diseases associated with hepatic dysfunction, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb), total bilirubin (TBIL) and direct bilirubin (DBIL). **Methods:** Internal quality control (IQC) data and related information of ALT, AST, Alb, TBIL and DBIL were collected by on-line questionnaire respectively in February, May and August of 2017. Cumulative coefficient of variations (CVs) were analyzed and the percentages of laboratories meeting the quality requirement were calculated in SPSS, which were calculated according to three levels of specifications for imprecision derived from within-subject biologic variation including the minimum, desirable and optimal specification. Chi-square ($\chi 2$) test was used to compare the pass rates among different analytes in 2017. **Results:** There are 1755, 1720, 1718, 1683 and 1583 laboratories submitting their

IQC data for ALT, AST, Alb, TBIL and DBIL, respectively. The percentages of laboratories meeting different imprecision specifications are shown in table below. For ALT, TBIL and DBIL, more than 80% participants obtained a satisfied Cumulative CVs no matter which imprecision specification applied. The acceptable rates of AST varied largely with different allowable imprecision. Alb had the best imprecision performance among all analytes, and even compared with the minimum criteria, the pass rate was only 60.7%. The percentages of laboratories whose cumulative CVs met three imprecision criteria for Alb were far less than the other four analytes (all P<0.01).

Analytes	Number of participant laboratories	Concentration of control materials		Cumulative CVs (%)		Percentages of laboratories meeting quality specification (%)		
		Median	IQC	Median	IQC	Minimum	Desirable	Optimal
ALT	1755	39.2	41.7	3.6	2.5	99.3 (1742/1755)	98.5 (1729/1755)	84.2 (1487/1755)
AST	1720	43.0	55.2	3.2	2.4	95.7 (1647/1720)	88.7 (1526/1720)	44.2 (726/1720)
Alb	1718	39.2	9.5	2.1	1.5	60.7 (1042/1718)	30.6 (525/1718)	3.4 (58/1718)
TBIL	1683	26.5	27.9	3.3	2.7	99.5 (1674/1683)	97.9 (1649/1683)	84.8 (1427/1683)
DBIL	1583	17.0	8.1	3.9	3.0	99.7 (1579/1583)	99.1 (1568/1583)	91.7 (1451/1583)

Conclusion: Although most of hepatic function tests acquired satisfactory imprecision performance and good IQC practice in 2017, only a few laboratories can pass the imprecision requirement for Alb. Therefore, clinical laboratories should pay more attention to the IQC of Alb to provide more accurate test results to patients.

B-174

One of these things is not like the other, or is it? Why laboratory informatics should lean heavily on lessons learned in disparate industries to create novel solutions.

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BACKGROUND: (study objectives, hypothesis, or a description of the problem) Often when trying to solve a problem, we find ourselves looking in familiar places for the answer. But when it comes to data and Informatics surface similarities aren't enough. Familiar methods such as HL7 and other health informatics go-tos have drawbacks that confine data entry to certain formats and prevent access to important non-standardized data sets stored in different formats. This technological set of road blocks can keep data in silos and prevent complete and accurate reporting as well as hinder case management efforts. METHODS: (study design and appropriate statistical analysis) A novel software framework built in C# with a WPS and React front end was modified from its original purpose to track trucking data to collect and store surveillance data from around the globe. This flexible software allowed for ingestion and normalization of various data types, creating a case management system that allow disparate data to be presented in normalized formats. In addition, because it was build for the trucking industry originally, the system was designed to adapt to large amounts of data that came from different locations, at different times, in different formats. RESULTS: (specific results in summary form) The software solution was adapted from its original purpose in trucking to intake surveillance and healthcare data from disparate sources (CSV, REDCap, XML, PDF, .JPEG, .PNG, and others), extract the data for input into SQL tables, combine the data into a case management system, and display data in a unified report format. The collected reports were then used to determine the cause of death in infant mortality cases globally. CONCLUSIONS: (description of the main outcome of the study) This project demonstrates how seemingly different problems -preventing childhood mortality and tracking trucks across the US - were solved using the same technical philosophy. We offer advice on how approaching technical puzzles from a different angle can lead to successful software projects as well as discuss the expertise, time, and budget needed to implement methods such as these.

B-175

Implementing procedure for laboratory critical values communication at the Hillel Yaffe Medical Center

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Objective: Laboratory critical values should be communicated immediately by telephone, throughout hospital departments. Oral communication is prone to errors, and therefore the "Read Back" procedure, is necessary. An interdisciplinary task force developed and implemented a structured procedure to maintain and improve communication between laboratory and clinical staff to improve patient safety. **Methods:** The team approved the laboratory critical values and wrote a procedure that included definition of critical steps in the process, staff role, and a reviewed form for process documentation. Laboratory and risk management members were assigned as process implementers. Critical values reports were produced by the laboratory and the team conducted tracers in the departments for matching data documentation. During departmental review, an immediate feedback and staff training was conducted by the team. Data analysis was performed monthly and transferred to the department heads emphasizing weaknesses and barriers. Longitudinal learning was achieved by staff meetings and publication of adverse events regarding critical values communication. **Results:** Data analysis showed a significant improvement in adherence to the "read back" procedure during the project period. In the ED a complete documentation was only 50% at the beginning and rose to 95%. Constant monitoring was required to maintain a high performance level. There were significant differences among the departments, depending on the management involvement in the process. **Conclusion:** Transferring critical information orally is necessary to ensure patient safety. The complex process requires a correct implementation and ongoing monitoring in order to ensure its proper execution.

B-176

Calculating the cost of poor quality: a multi-facility study

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Background: The Cost of Poor Quality (COPQ) concept was first described by Joseph Juran in 1951. COPQ can be defined as the cost of not doing something right the first time or "the costs associated with providing poor quality products or services". Although it is widely accepted that poor quality costs organizations significant amounts of money, postulated at 20% of sales for an average company, there is not much published work on COPQ in the context of the clinical laboratory. Another obstacle for application and adoption of the COPQ concept is that there is no standardized and widely accepted methodology to calculate COPQ. The COPQ concept is useful in demonstrating the financial value provided to a clinical laboratory or hospital by its quality program through cost avoidance and cost savings realized through elimination of root causes of nonconforming events. Without the intervention provided through the nonconforming event management system and quality improvement initiatives provided by the quality program, the laboratory and/or hospital would continue to experience financial losses for these events, in addition to potential patient safety risks. This study sought to develop a standardized tool incorporating feedback from leaders from multiple facilities in different geographical locations across the United States and across a variety of types and sizes of laboratories. **Methods:** A standardized COPQ worksheet, referred to as the COPQ Calculator, was developed and tested by seven leaders from multiple facilities across the US. Feedback was incorporated and the resulting COPQ Calculator was then deployed at the same seven facilities for a study on seven types of nonconforming events commonly encountered in the clinical laboratory: Specimen Mislabels, Instrument Downtime, Test Reruns, Proficiency Testing Failures, Corrected Reports, Product Recalls, and Turnaround Time Delays. The group met August 16th, 2017 in Indianapolis, Indiana for a full day to discuss use of the COPQ calculator and to calibrate their COPQ calculations. **Results:** All contributors to this study successfully utilized the tool to collect COPQ data for the seven types of nonconformities for the duration of the study. Data was collected from 6/27/2017 to 2/21/2018 and included COPQ calculations for 160 nonconforming events. Microsoft Excel was utilized to analyze the data. This poster presents COPQ ranges for each type of

event, as well as the median and average COPQ figures for each event type. **Conclusion:** This study succeeded in providing a free widely available, interactive tool for laboratory professionals to calculate COPQ as well as to provide COPQ figures for common event types that laboratory professionals can reference when articulating COPQ in their facilities. Understanding, articulation and examples of the COPQ concept in the clinical lab will help laboratories to gain financial investment and executive buy in for their quality programs. The COPQ data from the 160 nonconformities captured will be published and available for laboratorians to reference in order to articulate the financial implications of nonconformities, demonstrate cost avoidance and/or cost savings through quality initiatives and to aid in securing additional investment in quality for their laboratories.

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Internal Quality Control Analysis for Urinary Total Protein and Urinary Microalbumin from 2014 to 2017 in China

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Background: Quantitative analyzing of twenty four-hour urinary protein has an impact on diagnosis of renal diseases, while urinary microalbumin (mALB) is the preferred analyte for the early diagnosis and monitoring of diabetic nephropathy. This study investigated the internal quality control (IQC) practice of these two analytes from 2014 to 2017, to have a general picture of variation tendency. **Methods:** IQC programs for these two analytes were organized by NCCL in China, in April for each year. Information including cumulative coefficient of variation (Cumulative CVs) were collected via an on-line questionnaire. For urinary total protein, the percentages of laboratories meeting quality requirement were calculated according to three kinds of allowable imprecision specifications based on biological variation including the minimum, desirable and optimal performance specification. Since there is no biological variation data of urinary microalbumin, 1/3 allowable total error (TEa) and 1/4TEa defined by NCCL from 2014 to 2017, were taken as quality requirement. Chi-square ($\chi 2$) test was used to compare the pass rates among different years. All data were calculated by SPSS 20.0. **Results:** There were 147/110, 174/137,205/166 and 243/201 laboratories submitted their results of urinary total protein and urinary microalbumin, respectively. Only 146/108 laboratories continuously submitted of these two analyte. The percentage of laboratories meeting different quality specifications were shown in table. Although there was no statistical significance of each accepting rate from 2014 to 2017, the percentage of laboratories satisfying each specification were increased year by year, roughly. By 2017, over 90% of laboratories had cumulative CVs meet optimum performance specification of urinary total protein, while more than 80% of labs satisfied 1/4TEa. **Conclusion:** In general, IQC practice of urinary total protein and urinary microalbumin was satisfied. Even so, more efforts should be taken to continuously improve the IQC practice of urinary total protein and urinary microalbumin.

Analyte	Statistics		2014	2015	2016	2017	P value
Urinary total protein	Cumulative CVs	Median (%)	4.57	4.45	4.50	4.33	—
		IQR (%)	3.84	2.99	2.50	3.27	—
	Allowable imprecision specifications based on biological variation	Optimum performance (%)	78.08	89.73	90.41	91.78	0.940
		Desirable Performance (%)	86.99	97.95	97.95	100.00	0.785
		Minimum Performance (%)	89.04	99.32	98.63	100.00	0.847
Urinary micro-albumin	Cumulative CVs	Median (%)	5.09	4.99	4.19	4.95	—
		IQR (%)	2.88	4.07	4.24	3.33	—
	Quality requirement	1/3TEa(%)	81.48	91.67	91.67	92.59	0.924
		1/4TEa(%)	69.44	77.78	83.33	80.56	0.940

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Improving efficiency in ionic calcium measurements in the an emergency public clinical laboratory using good practices in the equipment management

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Background: The challenge of any large clinical laboratory is to keep the facilities and equipment available, with maximum reliability, within the best possible operating condition and using in maintenance management, which will enable the performance in the processes efficiency to support strategic decisions. This study aims to evaluate the impact of the equipment management (GE) on the efficiency of the ionic calcium measurement of a public, hospital and tertiary emergency laboratory, through the turnaround time (TAT). **Methods:** The study was performed between March / 2016 and April / 2017 in the emergency laboratory of a tertiary public hospital. For the measurements of serum ionic calcium was used the methodology Selective Electrode Ion (ICA-1) in ABL 800 Flex analyzer (Radiometer - Copenhagen). GE was structured using tools such as planning, FMEA, the PDCA cycle, the concepts of total productive maintenance, kaizen, 8S program, training and qualification of operators, employees as sector multipliers, computerized equipment management software, well detailed documentary, safe operation and set of performance indicators per equipment. The following indicators were studied and comparing with TAT: mean time between failures (MTBF), mean time to repair (MTTR), number of preventive and corrective maintenances, and percentage of availability. The Minitab v.15.0 software was used in the statistical analysis. This included: mean, standard deviation, median, normality test, trend analysis, Pearson correlation analysis. **Results:** The risks were assessed through the FMEA and action plans were implemented. The correct execution of the planning and the application of the mentioned tools allowed improvements. The investments in education were positive, resulting in an increase in the training mean from 1 to 2hr / employee / m. The implementation of the concepts of total productive maintenance with its eight pillars generated greater commitment of the operator with the equipment. There was a correlation between training hours and TAT reduction (r = 0.92).The relationship with the technical assistance team of the supplier was consolidated and promoting a faster service with a reduction in the MTTR of 75% (from 6 to 1,5 hr). The mean time between failures (from 32 days to 180 days) and the percentage of availability increased (from to 92% to 99.9%), a smaller number of stops of unscheduled equipment (n=2). The trends analysis confirmed this data. The number of complaints from the medical staff for delays in the delivery of reports decreased (from 33 to 1/ month).**Conclusion:** This approach adopted allowed increasing the efficiency in the serum ionic calcium dosages, reducing the non-scheduled stops and providing greater availability of the analyzer. It has contributed to increase the useful life of the equipment, bringing flexibility to production, knowledge of the machines by the operators, enabling innovation and cultural change.

B-179

Harmonization of the chemistry area in a network of clinical laboratories through the sigma metric and the comparison with percentiles

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Background
Achieving the harmonization and standardization of results in clinical laboratories is one of the challenges of laboratory medicine. Many organizations in the world have been created to pursue this goal. The Colsanitas Clinical Laboratories process an average of 873,141 tests per month, among 60 laboratories with 6 in emergency services, 12 for outpatient care, and one Referred laboratory center.
Objective
This work shows the harmonization result for 36 analytes of Blood Chemistry in a network of 15 laboratories with 21 measurement systems, during 2017.
Method
Pursuing harmonizable results, has been used several analytical quality assurance tools such: Definition of analytical performance limits as TEa, from different sources (BV, RilliBak and state of the art per percentiles), integrated QC graphs (Levy-Jennings and TE), monthly reports of Interlaboratory comparison, analytical sigma Metric of TE, SigET, Comparison by percentiles, "Performance-Per-Percentiles-PER3", statistical control rules and validation of analytical runs with sigma metric criteria, certification processes contribut-

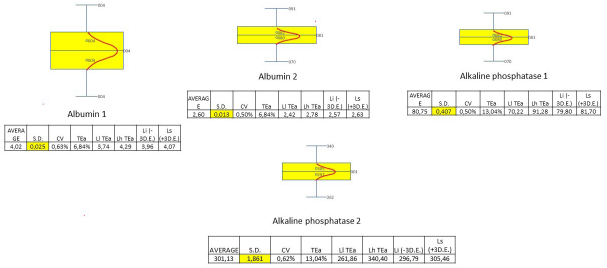
ing to improve the organizational procedures (training and continuing education)

Results

26 of the 38 analytes had a CV≤ 1%. The 96.75% had a ≥ 1.96δ, and the 63% ≥ 5δ . The 95% of the analytes were within the demanding analytical performance limits established by the laboratory network. The 49.4% of the tests were within the best performances of its peer group ($P_{10} \geq \leq P_{30}$), compared with an international network with Roche Cobas systems.

Conclusions

The use of tools as sigma metric, certification processes, contribute to the achievement of the harmonization of the results. It´s possible to keep continuously and consistently harmonized results with a high level of quality (High sigma metric) and stay consistently among the best performances in the world.



B-180

Sigma metrics for assessing the analytical quality of clinical chemistry assays: A comparison of two approaches

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Introduction: Two approaches were compared for the calculation of coefficient of variation (CV) and bias, and their effect on sigma calculation, when different allowable total error (TEa) values were used to determine the optimal method for Six Sigma quality management in the clinical laboratory. **Methods:** Sigma metrics for routine clinical chemistry testing using three systems were determined in June 2017 in the laboratory of Peking Union Medical College Hospital. Imprecision (CV%) and bias (bias%) were calculated for ten routine clinical chemistry tests using a proficiency testing (PT)- or an internal quality control (IQC)-based approach. TEa from the Clinical Laboratory Improvement Amendments of 1988 and the Chinese Ministry of Health Clinical Laboratory Center Industry Standard (WS/T403-2012) were used with the formula: Sigma = (TEa − bias)/CV to calculate the Sigma metrics ($\sigma_{CLIA,}$ $\sigma_{WS/T}$) for each assay for comparative analysis. **Results:** For the PT-based approach, eight assays on the Beckman AU5800 system, seven assays on the Roche C8000 system and six assays on the Siemens Dimension system showed σ_{CLIA} > 3. For the IQC-based approach, ten, nine and seven assays showed σ_{CLIA} > 3 on Beckman AU5800, Roche C8000, and Siemens Dimension, respectively . Some differences in σ were therefore observed between the two calculation methods and the different TEa values. **Conclusion:** Both methods of calculating σ can be used for Six Sigma quality management. In practice, laboratories should evaluate Sigma multiple times when optimizing a quality control schedule. **Figure 1** Comparison of σ_{CLIA} values calculated using two methods for the same test item. Note: 201721-201725 represent the lot number of proficiency testing materials, and 45751 and 45752 represent the lot number of Bio-Rad chemistry quality control materials. The short-dashed horizontal lines indicate the 3σ level line.

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Application of Sigma-metrics for assessing the analytical performance of clinical chemistry tests

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Background:

The core laboratory in Ng Teng Fong General Hospital, Singapore uses two ARCHITECT c16000 analyzers and one ARCHITECT c8000 analyzer connected to an integrated ACCELERATOR a3600 track system (Abbott Diagnostics, Chicago, IL, USA) for its clinical chemistry testing service. There is a need to objectively and quantitatively assess our laboratory's performance on 42 routine chemistry tests so as to provide a quality benchmark for the service provided to our clinicians.

Methods:

Using quality control materials and interlaboratory comparison data, bias and imprecision were calculated. Quality specifications were extracted from published sources for Total Error Allowable (TEa) such as CLIA, CAP and the Ricos Biological Variation database. A normalized method decision chart was generated and an average Sigma-metric was calculated for each test to assess the laboratory's performance.

Results:

The bias and imprecision were generally well within 30% of TEa, across the different instruments as well as concentration levels covered by the QC material. Of the 42 tests evaluated, 33 were operating at 6 Sigma (World Class performance), 6 were operating at 5 Sigma (Excellent performance), 1 was operating at 4 Sigma (Good performance), 2 were operating at 3 Sigma (Moderate performance), and none were operating at or below 2 Sigma (Poor performance).

Conclusions:

The Sigma-metric analysis showed that our laboratory has excellent performance, with 39 out of 42 (92.9%) tests operating at 5 Sigma and above and no tests below 3 Sigma. This high level of quality means that we can be confident that our laboratory

service is providing high quality results. This also means that we can seek greater workflow efficiencies and cost savings in our laboratory with a more optimized quality control strategy in the future.

B-182

The CDC's Laboratory Medicine Best Practices Initiative (LMBPTM): Systematic Reviews for Evidence-Based Laboratory Medicine Quality Improvement

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Objective: The CDC's Division of Laboratory Systems sponsors the Laboratory Medicine Best Practices (LMBP™) initiative, with a vision of evidence-based laboratory medicine quality improvement in support of health care and patient outcomes. The LMBP™ initiative uses a multi-stakeholder, six-step process termed the A-6 cycle.[1] This process has produced important best practice recommendations through a systematic review process that considers laboratory medicine quality improvement and quality assessment studies. Here we describe some of these achievements for increased awareness of the LMBP™ initiative. **Methods**: LMBP™ systematic reviews are done using the A-6 method developed by the CDC LMBP™ team, in collaboration with an external LMBP™ Workgroup, topic-specific expert panelists, and professional organizations (e.g. the American Society for Microbiology, and the American Association for Clinical Chemistry). Nationally important laboratory medicine quality gaps are identified, and relevant evidence is quality scored and synthesized for evidence summaries and practice recommendations. Evidence summaries include ratings on the strength of evidence as substantial, moderate, suggestive, or insufficient, and results from meta-analysis, with judgments on consistency of practice intervention effect across studies. Evidences for which the overall strength is substantial or moderate are used to develop recommendations. LMBP™ solicits topic suggestions for systematic reviews through their mailbox: LMBP@cdc.gov, and is refining a process for stakeholders to submit quality improvement/assessment data to the Initiative. **Results**: Since 2012, ten systematic reviews have been published, and 2 reviews are currently accepted for publication.[2] Six new reviews are in progress, while four reviews previously published are currently being updated with new evidence. **Conclusion**: The LMBP™ A-6 cycle is increasingly gaining success, defined as production of evidence-based findings with practical utility to laboratory professionals, completion of evaluations of recommendations in new settings, and strengthening laboratory services to achieve local quality goals with increased likelihood of improved health outcomes. To better achieve success, additional networks and stakeholder involvement are continuously sought in order to increase the relevance of LMBP topics, obtain unpublished quality improvement/assessment data, provide assistance in the process of evidence-based practice recommendation development and dissemination, and evaluate processes and outcomes of best practice implementation in various settings.

1. Christenson RH, Snyder SR, Shaw CS, et al. Laboratory medicine best practices: systematic evidence review and evaluation methods for quality improvement. Clin Chem. Jun 2011; 57(6):816-825.
2. Published systematic reviews and findings at https://wwwn.cdc.gov/labbestpractices/.

B-183

Creation of a Successful Professional Development Program for Women in a Major Medical School Pathology Department

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Introduction: According to 2015 AAMC data, the percentage of full professor faculty who are women in US medical schools is 22% and the percent full professor women within pathology departments is 26%. To improve promotion and retention of women in pathology, professional development for faculty and trainees at major medical schools is important. The percentage of women faculty at the full professor level at Washington University School of Medicine (WUSM) is below AAMC average at 19% and within Pathology is 13%. Hence, professional development for women within the Pathology Department of WUSM is particularly important. **Goals:** Our goal was to create a women's professional development program within the Department of Pathology at Washington University School of Medicine. **Methods:** The **"**Women of AP/CP", a forum for women faculty, residents, and fellows was created in November 2012. The forum meets at the University from 4:00-5:00 once per month and includes various topics, articles, books and invited speakers that cover a broad range of professional development subjects. After five years of activity, the success of the forum was assessed by surveying past attendees. A survey was created using Surveymonkey.com and sent to 65 women who had been invited to the forum over the five year period. Responses were received from 26/65 (40%) of women surveyed. **Results:** Junior faculty constituted 42% of attendees, with fellows (35%), residents (27%), mid-career (23%) and senior faculty (4%) attending in descending frequency. 100% of responders indicated that they found the content valuable to their professional development. Virtually all attendees agreed that including both faculty and trainees enhanced their experience, with only one faculty indicating they felt inclusion of trainees diminished the experience. 95% of respondents found the time of day for the program (4 to 5 pm) to be appropriate. The content topics rated as most useful were "Increased awareness of issues facing women in science" (95%), "Increased knowledge of faculty development programs and resources" (89%) and "Increased awareness of unconscious bias" (74%). A panel discussion with high profile successful women was the highest rated guest speaker event. "Career development strategies" was the highest rated topic covered. "Women Don't Ask" and "Ask for It" by Linda Babcock & Sara Laschever were tied for the highest rated books reviewed, and "Speaking out about gender imbalance in invited speakers improves diversity" (Klein R *et al*. *Nature Immunology* 2016;18:475-77) was the highest rated article covered in the forum. A session on promotion by the department chairman was rated highly. Questions such as "most valuable session" and "suggestions for future sessions" were also addressed. **Conclusion:** Here we describe the formation of a successful professional development program for women within a pathology department at a major medical school. The most valuable topics covered in this forum included unconscious bias and negotiation skills for women. This format could easily be replicated in any academic department. Knowledge of what topics women found most valuable can help direct the content and enhance successful outcomes of these types of professional development programs.

B-184

Application of Sigma-metric Run Size Nomogram to Establish Multistage Bracketed SQC of 8 Enzymes

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Background: Bracketed statistical quality control(SQC) was proposed in 2016 CLSI (C24-Ed4)document, for the purpose of reducing patient risks. Recently, Westgard has drawn a sigma-metric run size Nomogram, which can visually recommend quality control(QC) rules and QC frequency of different sigma test items, and came up with multistage bracketed SQC. In this study, we intended to evaluate the sigma performance of 8 enzymes and apply multistage bracketed SQC. **Methods:** (1) Sigma Performance Calculation: Calculate sigma levels using formula:*sigma=(TEa%-Bias%)/CV%,* where TEa is from the updated quality specification of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variation data issued in 2017, bias is obtained through external quality assessment (EQA), and CV is the coefficient of variation of internal QC data for consecutive 6 months. (2) Multistage bracketed SQC: Estimate the daily workload of each test item in our laboratory, and design the expected reporting QC intervals. According to the sigma-metric run size Nomogram drawn by Westgard, determine "start-up" and "monitor" QC rules of ALT, AST, GGT, ALP, LDH, CK, AMY, LIP based on different sigma performance. Design SQC schedules and apply them in our laboratory. **Results:** The sigma performance of 8 enzymes were 4.33, 4.89, 4.70, 2.76, 1.83, 9.72, 6.14, 3.35, respectively. For ALT, ALP, LDH and LIP with sigma less than 4.50, MR N4 QC rules were recommended in the whole QC schedule; For AST and GGT with daily workload of 1500-2000 and sigma closed to 5, MR N4 in the "startup" stage and 1_{3S} N2 in the "monitor" process were recommended. For CK and AMY with sigma more than 6, 1_{3S} N2 were suggested in the whole QC events. **Conclusion:** Multistage bracketed SQC is mainly determined by sigma performance, setting personalized multistage QC rules and frequency, which can monitor risks in the assay process and reduce patient harms. Nevertheless, utilization of tests with low sigma is costly and the best is to improve their sigma levels in the laboratory.

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Investigation and Analysis of Imprecision of Four Years' G6PD in Neonatal Screening in Clinical Laboratories in China

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Background: Glucose-6-phosphate dehydrogenase (G6PD) is an important constituent of neonatal genetic metabolic disease screening, G6PD deficiency is one of the major causes of neonatal hemolytic disease. The aim of this study was

to investigate and analyze the imprecision of internal quality control (IQC) of G6PD in neonatal from 2014 to 2017, in order to have an integral understanding of its imprecision level of measurement in Chinese clinical laboratories. **Method**: The laboratories' cumulative coefficient of variation (CVs) and relevant data of four years' G6PD were collected from the external quality assessment (EQA) software. Microsoft Excel 2010 and SPSS 20.0 were used to analyze the data. The one third and one fourth of allowable total error (10% and 7.5%) as well as quality specification based on biological variation including the minimal (5.5%), desirable (3.7%), optimal (1.8%) allowable imprecision were used to evaluate whether laboratories could satisfy the quality requirement and to calculate the percentages. **Results**: From 2014 to 2017, there were 69, 85, 101, 120 laboratories submitted their data, respectively. The results are shown in the table below. The majority of participant laboratories obtained satisfied CV when 1/3TEa was used (63.77% to 75.83%). About half of laboratories obtained satisfied CV when 1/4TEa was used (44.93% to 55.83%). The percentages of laboratories meeting quality requirements increased gradually from 2014 to 2017 no matter 1/3TEa or 1/4TEa was used. However, only a few laboratories could meet quality requirement when using the quality specification based on biological variation (<40%). **Conclusion**: Although the imprecision of G6PD's testing in China has been improved from 2014 to 2017, the overall level of measurement was not very good. Laboratory managers should highlight and monitor cumulative CVs of G6PD continuously. Meanwhile, it is suggested that more laboratories in China should participate in IQC program of G6PD, and make effort to improve the quality of measurement.

Item	Year	Number of participant laboratories	Cumulative CVs (%)		Percentages of laboratories meeting quality requirements (%)				
			Median	IQR	1/3TEa	1/4TEa	minimal	desirable	optimal
G6PD	2014	69	6.50	5.09	63.77	44.93	31.88	20.29	5.80
	2015	85	5.58	4.09	64.71	54.12	38.82	15.29	3.53
	2016	101	5.55	4.41	69.31	54.46	39.60	21.78	5.94
	2017	120	6.02	4.24	75.83	55.83	35.83	20.83	5.83

B-186

National survey on quality indicators related to the acceptability of samples in China

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Background: The quality of samples is crucial to ensure the accuracy of the test result. There are six quality indicators (QIs) related to the acceptability of samples applied in clinical laboratories of China, including incorrect sample type, incorrect sample container, incorrect fill level, anticoagulant sample clotted, haemolysed sample and unreceived sample. The aim of this survey is to investigate the status of the six QIs in clinical laboratories of China. **Methods:** A survey on 18 QIs was performed in 32 provincial clinical testing center all over China in 2017. Data of the six QIs were collected via Clinet-EQA reporting system established by National Center for Clinical Laboratories. The results were evaluated with sigma scale(σ) and percentage. **Results:** There were 7703, 7711, 7708, 7346, 7657 and 7620 laboratories summitting their data for the six QIs, respectively. The medians of the six QIs were 0.013%, 0.010%, 0.031%, 0.076%, 0.055% and 0.000%, and the medians of sigma value were 5.2, 5.2, 4.9, 4.7 ,4.8 and 6 in sequence. The detailed distribution was shown in the table below.

Evaluation index	Classification	Incorrect sample type		Incorrect sample container		Incorrect fill level		Anticoagulant sample clotted		Haemolysed sample		Unreceived sample	
		Number	Proportion (%)	Number	Proportion (%)	Number	Proportion (%)	Number	Proportion (%)	Number	Proportion (%)	Number	Proportion (%)
Percentage (%)	0-0.05	5148	66.83	5846	75.82	4566	59.24	3058	41.62	3698	48.30	7363	96.63
	0.05-0.25	1723	22.37	1472	19.09	2143	27.80	2767	37.67	2585	33.76	200	2.62
	0.25-0.45	412	5.35	229	2.97	442	5.73	705	9.60	645	8.42	30	0.39
	0.45-0.65	154	2.00	64	0.83	222	2.88	300	4.08	284	3.71	11	0.14
	0.65-0.85	84	1.09	32	0.41	90	1.17	149	2.03	147	1.92	7	0.09
	0.85-5	182	2.36	68	0.88	245	3.18	367	5.00	298	3.89	9	0.13
σ scale	0-3	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	3-4	281	3.65	109	1.41	361	4.68	546	7.43	482	6.29	17	0.22
	4-5	3053	39.63	2783	36.09	3871	50.22	4453	60.62	4486	58.59	448	5.88
	5-6	1287	16.71	1738	22.54	1237	16.05	445	6.06	1163	15.19	705	9.25
	6	3082	40.01	3081	39.96	2239	29.05	1902	25.89	1526	19.93	6450	84.65

Conclusion: The six QIs related to the acceptability of samples have played an important role in ensuring the quality of samples. Although the overall performance of the six QIs in 2017 is quite satisfactory, clinical laboratories should insist on monitoring the QIs and take more effective measures to reduce unacceptable samples.

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Laboratory Assessments: Management Of Non Conformities In 'Management Requirements, Clause 4' - Single Hospital Experience.

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Objective: To review the nonconformities (NC) reported on the management requirements clauses of ISO 15189, its occurrence and impact on improving the quality of laboratory services. **Background:** Most of the accreditation bodies assess and provide recognition to the quality and competence of medical laboratories based on ISO 15189 standards. The laboratories take appropriate corrective measures for the closure of reported non conformities resulting in compliance towards the accreditation standards. **Methodology:** Nonconformities reported during on-site assessments by the external assessment team from 2007 to 2017 were retrieved and tabulated based on the Main and Sub clauses of Management Requirements - Clause 4. Data was analyzed according to the number and nature of NCs reported and presented in the form of Number (count) and percentage by clause and sub-clause. It was reviewed for the frequency of occurrence, type and its impact on the Quality Management System (QMS). **Results:** The highest number of NCs was reported in Document control (21.1%); QMS and Evaluation and audits (13.2%) each; Organization and management responsibilities (10.5%) and 7.9% in Resolution of complaints and Control of records each. NCs under rest of the clauses were below 5.3%. Of the total number of 38 NCs, it was observed that there were equal number of (19) Major and Minor type. **Discussion:** The accountability, coordination and execution of quality related activities across the laboratories were enhanced through defining roles of deputies and laboratory Director. The in-house external assessors' induction into the internal audit programme added value to the audits. Sample acceptance criteria and TAT defined with clinician's concurrence were among many other quality indicators that were adopted for continual improvement. The implementation of vendor evaluation system controlled the external services and supplies, providing continuous monitoring of vendor services quality. A mechanism was evolved through biomedical unit for monitoring of equipment. Safety devices such as eye wash stations were installed, staff education were addressed through regular CMEs. Version controls, review processes, identification of obsolete documents and uniformity in documents and records were achieved through structured formats and master files. The above changes undertaken to achieve compliance have impacted in enhancing the Laboratory leadership, internal audits, continuous education, safety, quality indicators, Document control and purchase procedures. **Conclusion:** It is evident from the above study that the NCs have reduced significantly over the decade. The number of NCs reported in 2008 (10) were reduced to four in 2017. This indicates that the assessments ensure quality and are a path for continuous and overall improvement of laboratory services. Ho and Ho (2012) in their study have similar observations indicating improvement through compliance with ISO 15189. Ref: Ho B, Ho E. (2012) - The most common nonconformities encountered during the assessments of medical laboratories in Hong Kong using ISO 15189 as accreditation criteria, Biochem Med (Zagreb). 2012;22(2):247-57. PMCID: PMC4062334

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Identifying Barriers in the Dissemination and Adoption of Clinical Laboratory Practice Guidelines (LPGs) and Improving the LPG Lifecycle

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Background: The Centers for Disease Control and Prevention (CDC) and the Clinical and Laboratory Standards Institute (CLSI) launched a study in 2013 to identify strategies to increase the dissemination and adoption of CLSI's laboratory practice guidelines (LPGs). CLSI selected two guidelines for this study, POCT12-A3, *Point-of-Care Blood Glucose Testing in Acute and Chronic Care Facilities*, and POCT13-A3, *Glucose Monitoring in Settings Without Laboratory Support*, because they are related to diabetes control, an important public health issue. **Methods:** A Web-based survey was distributed to gather opinions about the selected LPGs' content, adoption, and implementation. The survey was designed around Cabana's framework (Cabana, et al. *JAMA*. 1999;282(15):1458-1465) concerning why clinical practice guidelines are not always followed. The survey assessed the following barriers: lack of awareness, external barriers, lack of outcome expectancy, and guideline-related issues. The survey was distributed to approximately 15,377 potential users of POCT12 and 14,250 potential users of POCT13 contacted through relevant professional organizations, mailing lists, and the Clinical Laboratory Improvement Amendments (CLIA) database. Additionally, the project team used the National Academy of Medicine (NAM) standards for clinical practice guidelines and the AGREE II tool to evaluate CLSI's LPG lifecycle and suggest improvements for the development and delivery of CLSI's LPGs. **Results:** 879 usable survey responses were obtained; yielding a 3% response rate; thus, potential response bias must be considered. Data reported here primarily pertain to POCT12, because few respondents were familiar with POCT13. Most respondents from the typical CLSI target audience were aware of CLSI, while responses from the non-laboratory-based respondents showed a lack of awareness. Responses from both audiences demonstrated a lack of awareness of these glucose testing LPGs. Additional reasons for not using these CLSI LPGs were external barriers (lack of educational materials/tools to facilitate implementation, added expense for the laboratory, staffing burden, and purchase price), lack of outcome expectancy (no perceived improvement to the current procedure), and guideline-related issues (LPGs are not easy to use and not written at a targeted end-user reading level that facilitates staff training, and the recommendations are not practical to implement). Evaluation using the NAM guidelines and AGREE II tool resulted in 15 recommendations for improving three key areas of CLSI's LPG lifecycle: committee formation, project idea generation and approval processes, and the document systematic review and revision process. Metrics were developed for CLSI to measure the success of the recommended process improvements to be implemented during the final year of the project. **Conclusion:** CLSI can increase uptake and use of LPGs by supporting products that facilitate the implementation of LPGs, developing LPGs that are more easily understood, and enhancing communication with the intended audience of its LPGs. These actions should help to overcome some of the barriers identified by the survey responses. Additionally, CLSI should consider restructuring LPG pricing. The identified lifecycle process improvements should enhance the quality and timely delivery of LPGs.

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Impact of cross-sex hormone therapy on common laboratory tests in transmen and transwomen

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Background: Reference intervals describe variation in healthy individuals so that pathologic values can be distinguished from normal physiologic values. For transgender individuals taking cross-sex hormone therapy, changes in physiology is expected, but the effect on laboratory tests is scarcely studied and cannot be easily predicted. Laboratory values that are out of range could initiate unnecessary diagnostic work up and alternatively, abnormal values may go unrecognized. **Objective:** This study aimed to determine alterations in common laboratory tests in a large, diverse population of transgender patients transitioning with CSHT. **Methods:** Retrospective data from a community transgender clinic and large county hospital was performed from 2007 to 2017. Lab values for complete blood count, complete metabolic panel, and lipids were recorded. We compared laboratory parameters of female to male transgender (FTM) and female transgender (MTF) patients on CSHT for > 6 months (median 21 months) to values of transgender patients assigned male (M) or female (F) at birth not taking CSHT. We excluded patients with recent illness, liver or kidney disease, non-fasting status (glucose >200 mg/dL or triglycerides >400 mg/dL), or non-compliance with CSHT. **Results:** We identified 264 unique patients (156 transwomen and 108 transmen) that met the inclusion criteria. 89 FTM and 133 MTF patients on hormone therapy were identified. Further, 87 transgender patients assigned male (M) at birth and 62 transgender patients assigned female (F) at birth not taking CSHT were identified for comparison. Transgender men: Transgender men (FTM) taking intramuscular testosterone for CSHT were found to have increased RBC, Hgb, Hct, creatinine, and ALT, while albumin and BUN decreased compared to F patients not taking hormone therapy ($p<0.005$). High-density lipoprotein values decreased significantly while triglyceride values increased on the lipid assessment ($p<0.005$). Although these patients were on hormones for various time periods, we showed that these changes were dynamic for the first 6 months, after which the values remained stable. Interestingly, a subset of patients (19%) had Hgb values $\geq$ 16.5 g/dL and/or hematocrit values $\geq$ 49% (Male reference interval: 40-54%, Female reference interval: 36-46%), values which might prompt consideration of polycythemia vera in cisgender men. Transgender women: Transgender women (MTF) taking either intramuscular or oral estradiol displayed decreased RBC, Hgb, Hct, creatinine, ALT, albumin, total protein, calcium, alkaline phosphatase, total bilirubin, and sodium, while glucose, and platelets were increased compared to M patients ($P<0.005$). Notably, no lipid parameters were significantly changed. **Conclusion:** Our results demonstrate substantial differences in several lab indices for FTM and MTF patients. These findings have important implications for interpreting lab tests. For instance, altered hematocrit or creatinine could prompt unnecessary work up for polycythemia vera and kidney injury or could prevent proper diagnosis of anemia or kidney disease. Alterations in lipid profiles in the context of testosterone can have potential implications in the assessment of cardiovascular risk. These findings underscore the need for transgender specific reference intervals for laboratory testing.

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Comparison of rates of nearly simultaneous, identical central laboratory testing associated with blood gas/electrolyte/metabolite point of care testing in two adult intensive care units

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Background: The GEM 4000™ (Instrumentation Laboratory, Waltham MA.) and ABL 800 (Radiometer, Copenhagen, DE) are point-of-care analyzers, primarily used in critical care settings to measure blood gases, glucose, and electrolytes. Previous studies have shown that the GEM 4000 produces borderline low sigma tests results compared to the ABL 800. While physicians using a low sigma analyzer have recourse to retesting with point of care testing, they might simply send blood to the central laboratory. In this study, we compared the number and costs of replicate tests sent to the central laboratory within 30 minutes of running the point of care test panel. **Methods:** Laboratory databases were mined for measurements of glucose and electrolytes using either twin GEM 4000™ instruments or twin Radiometer 800 ABL blood gas analyzers at either the Foothills Hospital adult ICU in Calgary, Alberta or the General Systems adult ICU at University Hospital in Edmonton, Alberta, respectively, between 2013-2016. We counted any concurrent testing (within $\pm$ 30 minutes) performed by the central laboratory Roche Cobas 8000™ or the Beckman DXc chemistry systems in Calgary and Edmonton, respectively. In Alberta, individual electrolytes and glucoses have been costed at $5.00 per test. **Results:** Each patient with GEM 4000 testing averaged 6.1 central laboratory sodiums, compared to the average patient with ABL 800 testing who averaged 2.5 sodiums. For Na, Cl, bicarbonate and potassium, the rate of central laboratory testing for the GEM patients was roughly 2.2 to 2.4 times that of the ABL 800. The yearly total cost for repeated central laboratory Na, Cl, bicarbonate, potassium and glucose testing was $120,000 for the GEM patients and $45,500 for the ABL 800 patients. **Conclusions:** One of the hidden costs of using a low sigma analyzer may be the increased costs of redundant central laboratory testing. In addition to this extra testing which is presumably associated with the use of a low sigma analyzer, other less tangible factors should be considered when selecting an ABG analyzer including the cost of diagnostic error.

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Impact of biochemistry critical values communication in outpatient outcomes

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Background: The effectiveness in the report process of critical values (CV) is considered an essential quality indicator in clinical laboratory to ensure patient's safety. In our Laboratory, there is a specific protocol for CV management, which sets up the communication pathway to properly inform these results to clinicians.The aim of this study is to evaluate the impact of communicating CV on outpatient's care. **Methods:** When a CV appears, date, time, laboratory results and person notified are recorded. Our biochemistry CV alert list is collected in the Table 1. We retrospectively evaluated the CV records and the consequent clinical actions undertaken from outpatient serum samples between January 2017 and October 2017. **Results:** A total of 100 CV corresponding to 89 outpatients were registered (Table 1). Regarding to actions triggered, patients were admitted into the Emergency Department (ED) in 41 CV events; visited in Day Hospital the same day of the phlebotomy in 16 and managed safely in their homes in three CV events. A total of 42 CV events corresponded to hypoglycemia, most of them seen in diabetic patients and only in two a medical action was registered. Therefore, in 57 from 60 CV (95%), their notification resulted in treatment initiated.

Table 1- Number of critical values, number of outpatients and number and frequency of events triggered in patient's clinical evaluation.

Test	Critical values (n)	Patients (n)	Events in which patients were visited in Day Hospital or ED n (%)
Potassium > 6,5 mmol/L	34	29	32 (94.1)
Potassium < 2,5 mmol/L	3	3	3 (100)
Glucose < 50 mg/dL	42	40	2 (4,8)
Calcium < 6,5 mg/dL	14	12	13 (92.9)
Calcium > 13 mg/dL	5	3	5 (100)
Sodium < 120 mmol/L	2	2	2 (100)
Total	100	89	57

ED, Emergency Department.

Conclusions: Although hypoglycemia was the most frequent CV event, its notification had a low impact on patients care. These results lead us to raise whether decrease the critical limit or not to notify this CV in case of diabetic patients. For the rest of CV studied, their notification resulted in treatment initiated in 95% of the outpatients affected. This data proves the relevance of informing life-threatening results for the safety of outpatients, in which ongoing health care is not provided.

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Apply learning analytics to teaching clinical chemistry via an online digital learning platform

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Background: Digital learning tools are widely adopted in current academic teaching. In addition to serving as effective platform for distributing materials, initiating surveys, and gathering teaching feedbacks, a well-designed learning module can also be used to study and understand the students' learning activity, study pattern, and their associated outcomes by tracking their log-ins and accesses to the course contents on the platform. **Objective:** The study is aimed to apply learning analytics to the teaching of two undergraduate level clinical chemistry courses: a lecture-based theory course and a laboratory-based practical one. The study tracks the pattern of the students' learning activity via its online learning module Blackboard Learn™ and compares their learning behaviors between the two different knowledge delivery methods. **Method:** The two courses are taught on a weekly schedule in the 17/18 Spring semester to 52 undergraduate medical laboratory science students. The students are instructed to study the course contents through the online platform Blackboard in addition to attending lectures and laboratory sessions. The content includes lecture notes, additional reading, and self-practice clinical cases. To track the students' learning patterns, we track and record their accesses to different components of the course contents, class announcement, and student surveys. To establish their study pattern, the date and time of their log-ins and number of downloads and on-line viewings have been analyzed in both the theory and practical courses. **Results:** The data were analyzed at week 6 of the semester, and all the students (n=52) had logged onto the learning platform for both courses. The average number of accumulative accesses to the course content for each student is 25 times for the laboratory course and 20 times for the lecture component. There are varying degrees of content access among the student group, with the highest over 80 times for some students, whereas the lowest less than 10. The standard deviations (SD) for the access clicks are 13 and 14 for the laboratory and lecture course respectively. When analyzed against the date and time, the content viewing from the students frequently peaks (60-70 accesses/day for the laboratory course, 40-70/day for the theory course) the day before the relevant lecture or the laboratory session takes place, even though the course content is normally made available 7 days prior to the session. Similarly, the number of log-ons also increases significantly before the submission deadline of each coursework assignment. The content access can be attributed to each participating student to establish an individualized study pattern. As the overall access for the practical course is higher than the tutorial theory course, the same study behaviors apply to most of the students too. There is a strong positive correlation (r^2=0.68, k=0.84) between the numbers of overall access times for the laboratory course and the theory course. **Conclusion:** The learning pattern of students can be reflected by their accesses to the online learning platform. Its correlation with teaching outcomes (grades and teaching evaluations) can be further explored to improve knowledge delivery and engage students participation.

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Anchoring Method Performance Evaluations with the Sigma Calculation De-emphasizes the Question: Is the Assay Fit for Purpose?: How much better are 10 sigma than 5 sigma (or 5 versus 2) assays?

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Introduction: In a recent paper, we compared the sigmas of two point of care blood gas/electrolyte analyzers. While one analyzer's sigmas were significantly lower, we were hard pressed to coherently express, based on sigma, one analyzer's analytical advantages over the other. This poster presents our investigations in exploring the putative advantages of a higher sigma. **Materials and Methods:** We used estimates of the biologic variation (BV) and analytical variation (AV) of the two analyzers (Table) to determined sigma. As clinicians primarily classify normal and abnormal based on institutional reference intervals, for both analyzers, we simulated, charted and compared serial patient data that were close to the upper and lower reference limits. We identified problematic analytes using Cotlove's and Harris'criteria: those analytes whose AV contributes more than 12% to the overall patient variation. **Results:** The Table shows the AV contribution to the total patient variation. With the exception of iCa, the simulated serial patient data generated for both analyzers are very similar when evaluated with typical reference limits. **Discussion:** The concentrations provided by the two analyzers are very similar from the view of a clinician. With the exception of iCa, nearly identical clinician diagnoses and therapeutic decisions would arise from either system. For iCa, the AV in the low sigma analyzer doubles the patient variation and obscures or even invalidates iCa interpretation. We propose that tests with a sigma value as low as 2 can be used for point-of-care, as they meet the 12% cutoff, and can be readily rerun if their values are unexpected. Tests with sigma as low as 2 produce acceptable results, and beyond the value of 5, sigma does little to denote quality in this setting. Clinicians using tests with sigma under 2 should be made aware of this AV issue.

Test	Patient BV	Inst1 AV	Inst2 AV	Inst1 Sigma	Inst2 Sigma	Inst1, AVcontrib	Inst2, AVcontrib
pH	0.02	0.001	0.010	20.0	2.0	0.1%	11.8%
K, mmol/L	0.2	0.02	0.04	10.0	5.0	0.5%	2.0%
pO2, mmHg	15.0	1.5	5.0	10.0	3.0	0.5%	5.4%
pCO2, mmHg	2.0	0.35	1.3	5.7	1.5	1.5%	19.3%
Glucose, mmol/L	0.5	0.1	0.4	5.0	1.3	2.0%	28.1%
HCO3, mmol/L	0.8	0.2	0.6	4.0	1.3	3.1%	25.0%
iCa, mmol/L	0.015	0.004	0.030	3.8	0.5	3.5%	123.6%
Cl, mmol/L	1.0	0.4	0.5	2.5	2.0	7.7%	11.8%
Na, mmol/L	0.5	0.5	0.8	1.0	0.7	41.4%	80.3%

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Four years microbiological laboratory activity summary of high volume Central Laboratory of Turkey

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Background: Cost reduction is the primary driving force of the healthcare reform; to survive, laboratories must adapt. The other benefits targeted are to use the recent technologies to decrease turnaround times, standardize the interpretation and reporting for cultures while reducing the staff needs. Therefore a Central Laboratory was designed for maintaining high quality services and cost effectiveness of consolidating separate Clinical Microbiology Laboratories in 11 hospitals (totally 3614 beds capacity) in Istanbul in 2014. This Central Laboratory has been serving to 13 hospitals (4972 beds capacity) since 2017. Nowadays approximately 430.000 samples are accepted and 2.400.000 tests are run per month in the Central Laboratory. In this study, we evaluated the laboratory based reflection of increasing work volume within three years on a test basis.**Methods:** The Central Laboratory test numbers (urine culture, antibiotic susceptibility tests, ELISA, serologic and PCR tests) between 2014 to 2017, were retrospectively evaluated from the received data with Automated Laboratory Information System (Ventura,Turkey) and statistically analysed.**Results:**Between the years 2014 to 2017, the total numbers of hospital bed capacity Central Laboratory served, has increased 37%. There are no changes in the number and the capacity of the devices. The number of; manual serologic testing has increased 33%, antibiotic susceptibility test has increased 32%, PCR has increased 30%, urine culture has increased 27% and the number of patients to be studied has increased 17%. The number of laboratory technician in the Central Laboratory decreased 12% and the number of Laboratory specialist increased 30% within the same time period. **Conclusion:** Despite the increased number of patients, and the decreased number of laboratory staff over the years, thanks to rational laboratory management in the Central Laboratory, the number of devices has remained same. Despite the increase in served bed capacity, number of tests we run, has not increased proportionally. Effective laboratory usage rules and hospital automation systems; such as the prevention of panel test requests, the organization of test request pages, visual warnings to the clinicians regarding the test request frequency, and the intermittent trainings to the clinicians has helped us to achieve this successful result.

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Performance comparisons among homogeneity and two kind heterogeneity systems used in laboratories

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Background: Measurement systems adopting instruments, reagents and calibrators manufactured by the same manufacturers are called homogeneity systems, and those that don't meet the requirement are heterogeneity systems. Currently the ratio of homogeneity to heterogeneity systems used by laboratories across China is about 1:1. Owing to a more open market, cost-effectiveness and convenience, more laboratories may tend to choose heterogeneity systems in the future. We here compared the performance of heterogeneity systems with homogeneity systems. **Method:** Part of National category 1 serum uric acid external quality assessment(EQA) data in 2017 were extracted, in this EQA program commutable materials with target value (416.6μmol/L) assigned by reference measurement procedures were repeatedly measured by all participants. After removing outliers by 3SD, there were 75 homogeneity systems (8 Abbott, 55 Beckman,12 Siemens) and 126 heterogeneity systems classified into two categories that systems adopting reagents and calibrators from the same manufacturers were classified as group 1 and systems adopting instruments, reagents and calibrators all from different manufactures were group 2 (group 1: 8 Abbott, 20 Beckman, 31 Hitachi, 10 Siemens; group 2: 3 Abbott, 22 Beckman, 22 Hitachi, 10 Siemens). Bias and inter-lab CVs were compared with biological variation data (bias<4.87%, CV<4.30%) as criteria for evaluation. **Result:** As shown in Fig1, for all 4 mainly used instruments, all homogeneity systems showed desirable bias within biological variation criteria and better inter-lab CVs compared with heterogeneity systems, but both homogeneity and heterogeneity systems were within CLIA88 requirement (17% bias). All systems showed inter-lab CVs within biological variation criteria, meanwhile, group 1 heterogeneity systems showed slightly better inter-lab CVs and better bias except Hitachi than Group 2. **Conclusion:** Homogeneity systems showed better performance than heterogeneity systems, and group 1 heterogeneity systems showed no obvious better performance than group 2.

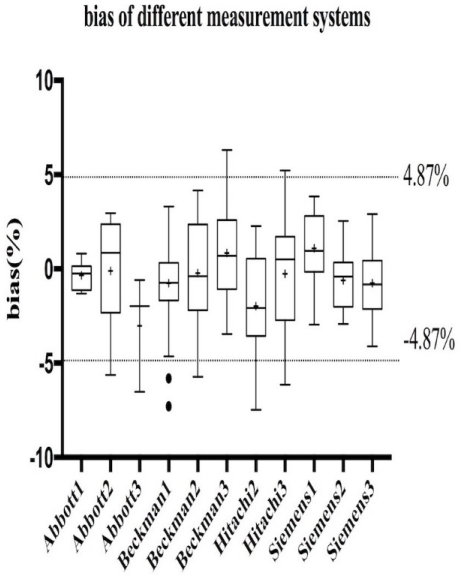

Fig1. code 1,2,3 separately represent homogeneity systems, group 1 heterogeneity systems and group 2 heterogeneity systems.

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Toward clinical laboratory productivity gains: machines or lean manufacturing?

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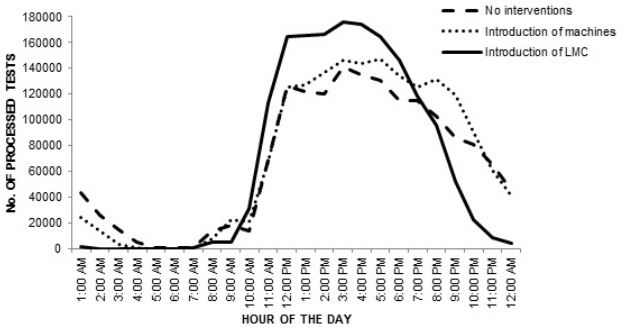
Background: Here, we evaluate the impacts of additional machines introduction and the lean manufacturing concepts (LMC) application into a pulled biochemistry and immunohormones production line of a large clinical laboratory located in Brazil's center-west. **Methods:** The analyzed laboratory sector consisted of integrated biochemical/immunologic unit composed of 10 ADVIA Centaur XP®, 04 ADVIA Chemistry 2400® and 04 IMMULITE 2000® coupled into the 70 meters Siemens Aptio®, approximately. The studied period was from January to June 2017 and was divided into three identical time frames: January and February (no interventions), March and April (introduction of machines), and May and June (introduction of LMC). On March, 2 ADVIA Centaur XP® and 2 IMMULITE 2000® were introduced. In May, LMC was introduced. LMC included in loco process flow mapping, time-motion study, and production capacity analysis. Measured variables were: the maximum tests processed per hour and turnaround time (TAT), related to productivity, and employee overtime, blood redraws, report corrections, related to defects. Calculations considered the variable average normalized by the number of blood tests performed on each analyzed period. **Results:** Compared to the no interventions period, the introduction of machines reduced: the TAT in 5%; the blood redraw in 16%; and the employee overtime in 22%. On the other hand, the maximum tests processed per hour increased in 1% and report correction in 25%. Compared to the introduction of machines period, the introduction of LMC reduced: the TAT in 25%; the employee overtime in 33%; the blood redraws in 17%; and report correction in 74%. In addition, the maximum tests processed per hour increased in 21%.



Conclusions: Increasing the number of machines brought more considerable gains to the variables related to the defects, but not productivity. Conversely, the LMC brought significant benefits to both productivity and defects variables (which are the crucial points of the Lean Manufacturing philosophy).

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Utilization of Laboratory Testing Algorithm for Celiac Disease in a Pediatric Hospital

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Background-Increasing prevalence of celiac disease (CD), primarily in the pediatric population, results in lifelong treatment and complications in a growing number of individuals. At Texas Children's, during our annual review of our send out tests, we found numerous celiac tests were ordered from a wide range of non-specialty health care providers. The Celiac Disease Foundation and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) implemented guidelines for assisting health care professionals by using a screening approach and standardizing clinical practice. Biopsy remains the gold standard for diagnosis of celiac disease but is associated with high cost, turn-around time, and risk. Herein, our main goal was to improve celiac disease test utilization, so we implemented an in-house celiac disease screening cascade and reflexed it to a comprehensive celiac testing panel if an abnormal screen result was obtained. **Methods-** We carefully analyzed all orders for comprehensive celiac testing (which includes genotyping) from July 2016 to September 2017 in the central laboratory at Texas Children's Hospital. We also examined the proportion of ordering providers that were gastroenterologist versus non- gastroenterologist health care providers. We initiated a screening cascade for celiac disease screening. Then, we conducted pathology review of all comprehensive celiac testing orders and made a recommendation based on patient's history to either send to comprehensive celiac testing or to change the standing order as an add-on to the in-house celiac screen. After the appropriate test was ordered, results were re-evaluated. **Results-**The total volume of celiac test orders for approximately 14 months period is 60 orders (n=60). The ordering physicians were gastroenterologist in 4 of the cases and a non-gastroenterologist in 56 cases. Out of 60 cases, there were only 3 of 60 cases that were approved for send out for comprehensive celiac testing, and 52 of 60 cases were altered to celiac screen from comprehensive testing. Subsequently, 5 out of 60 cases were tested further due to incorrect ordering. Only 1 of the 52 celiac screen were positive and reflexed to the comprehensive panel. Remaining 51 out of 52 resulted as negative celiac screen. Over 14 months, this strategy resulted in direct cost savings of approximately $117,550. We were also able to improve turnaround time for effective management and treatment of these patients. **Conclusion-** Our data reflects utility of celiac disease test utilization; by effectively implementing strategies to modify physicians ordering pattern and by advocating celiac reflexive cascade, we were able to improve celiac disease test utilization, reduced cost of testing and improve turnaround time for effective patient management. Test utilization strategies that are initiated with collaboration of pathologist with their clinical counterparts in every area of medicine will improve clinical management of patients effectively.

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The Use of Proficiency Testing Results for Predicting Laboratories' Future Risk for CLIA Deficiencies

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Background: The Clinical Laboratory Improvement Amendments of 1988 requires approximately 35,000 U.S. clinical laboratories to participate in proficiency testing (PT) for > 80 specified clinical tests. Centers for Medicare & Medicaid (CMS) necessitates that these laboratories receive biennial on-site inspections. If the inspectors identify unsuccessful PT performance, they cite the mandatory condition deficiency tag (D-tag) D2016. Condition D-tags indicate an erroneous practice requiring correction for the laboratory to continue testing. We hypothesized that PT scores may be a leading indicator to predict risk of future inspection deficiencies, because laboratories cited with D2016 would need to demonstrate improved testing quality before the next inspection compared to laboratories with one-time unsatisfactory (less than 80%) and marginal scores (80%). This study investigates the use of PT performance, including unsatisfactory and marginal scores, to identify risks of more serious quality problems. **Methods:** The analysis used PT data (three events/year) and D-tag data from Certificate of Compliance laboratories included in the CMS Quality Improvement and Evaluation System database. Data from 2007-2016 were divided into five periods of two years each. Analysis of the association between the number of condition deficiencies in the next two years and whether a laboratory received a D2016 citation utilized Chi-square tests. Analysis of variance (ANOVA) tests assessed the differences between the mean number of condition deficiencies from unsatisfactory (≤ 60%), marginal (80%), and perfect (100%) PT scoring groups. All tests were two-sided and a two-sided p-value of <0.05 or a 95% CI not containing 1.00 was considered statistically significant. **Results:** When a laboratory received a D2016, it was 11 times more likely to have fewer non-PT related condition deficiencies in the next inspection. The odds ratio decreased to 9.7 after controlling for the number of analytes that require PT. Comparisons of the mean numbers of deficiencies for unsatisfactory, marginal, and perfect PT score groups, with and without D2016, between inspection intervals were statistically significant at the p=0.05 level except for the perfect score group without a D2016. We repeated the analysis with refined score groups to separate one unsuccessful or marginal score versus two or more. The change in the means from one inspection period compared to the next for the two or more marginal score group was 0.31. For all other groups the change ranged from -0.12 to -5.73. **Conclusion:** Our results suggest that unsuccessful PT performance (D2016) may be used to predict a laboratory's future performance. Laboratories with a D2016 deficiency were at a lower risk of receiving condition deficiencies cited in the next inspection compared to laboratories without D2016. Laboratories without a D2016 citation but with two or more marginal scores in a 2-year period were more likely to have condition deficiencies cited in subsequent inspections, suggesting successful PT performance with marginal PT scores increases future risk of receiving condition D-tags. This implies marginal PT performance is an actionable leading indicator of quality problems. Further studies will include additional variables including number of PT analytes, non-PT D-tags, and PT-related D-tags.

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Our New-Resident Trainee Educational Intervention Efforts Play a Significant Role in Reduction of Inappropriate Laboratory Add-on Testing Orders

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Background: Since our hospital's Meditech system lacks a sophisticated rules-based physician order entry system, we sought to improve add-on test (AOT) order accuracy through using resident training initiatives. An AOT is an order for a test to be performed on a specimen already collected for previous testing purposes. While AOT helps reduce new specimen collections on patients and can assist in the reduction of iatrogenic anemia or infection, several issues have challenged our laboratory in handling inappropriate AOT orders from our new resident physicians. The majority of our inappropriate AOT orders include: 1) a duplicate order for a test already performed, 2) the order is added on to a wrong specimen sample type and/or collection tube/container, 3) specimen instability issues for the add-on analyte requested, and 3) the remaining quantity of specimen is not enough for the new add-on order. Methods: We performed two studies with each using AOT data extracted from our Meditech laboratory information system in a one-week period in November, 2015 and August, 2016. The 2015 study served as a baseline for the follow-up study conducted one month after an educational initiative for new medicine residents

to improve the quality of AOT ordering at the beginning of the academic year in July, 2016, including introducing our specimen collection "pocket" tube guide and specimen collection requirements. Also, an order-entry alert was added to the AOT order module when ordering a test listed on our " inappropriate AOT" test list. We examined AOT patterns including ordering physicians, total number of AOTs, number and types of performed AOTs as well as the non-performed or rejected AOTs. Chi-square was used with significant criteria of $p < 0.05$. Results: We observed significant decreases in the total numbers of ordered AOT (941 to 874, p=0.001) and the rejected AOT (161 to 116, p= 0.027). However, within the latter group, we did not observe a statistically significant reduction in the total number AOT rejections for duplicate orders (p= 0.443), wrong specimen collection tube/container (p=0.597), quantity not sufficient, and/or specimen stability AOT rejections (p = 0.999). Conclusion: It appears that our resident educational intervention could have played a significant but relatively minor role in the improvement of AOT issues in terms of 1) reducing the total number of AOT and 2) the number of problem AOT and 3) perhaps shifting add-on testing over to increased tests ordered on the original test request.... yet another desirable educational outcome. We did not however observe a statistically significant reduction in the number AOT rejections for duplicate orders, wrong specimen collection tube/container, quantity not sufficient, and/or specimen stability AOT rejections. We therefore conclude that these latter issues cannot be effectively handled without the use of a sophisticated, rules-based expert physician order entry computer system that alerts the new resident physician interactively at the time of order when an AOT is not possible.

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Comparison of Proficiency Testing Performance for Analytes of a Complete Metabolic Panel: Hospital Laboratories to All Other Testing Sites

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Background: Clinical laboratory technologies and testing methods are changing at a rapid pace. Proficiency testing (PT) is an important tool for monitoring test performance, detecting and identifying problems, and improving laboratory quality. Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), laboratories performing nonwaived testing on patient samples are required to participate in PT. The majority of PT analytes are tested by clinical laboratories at a frequency of five challenges per event and three events per year. A testing event score of at least 80% is needed for a laboratory to be considered satisfactory for each PT analyte. This means that at least 4 out of 5 results per event must be within the CLIA criteria of acceptable performance for that analyte. Failure to achieve 80% in 2 consecutive events or 2 out of 3 events results in unsuccessful performance for that PT analyte. A complete metabolic panel (CMP) measures kidney and liver functions as well as electrolyte levels, and is one of the most frequently ordered laboratory test panels. The 14 analytes measured in this panel include alanine amino transferase, albumin, alkaline phosphatase, aspartate amino transferase, bilirubin, blood urea nitrogen, carbon dioxide, chloride, creatinine, glucose, calcium, potassium, sodium, and total protein. All are CLIA-required PT analytes. Most hospital laboratories and independent laboratories (HI) were required to perform PT prior to the implementation of CLIA and were shown to have lower PT failures rates than all other testing sites (AOT) (e.g. long-term care facilities, nursing homes, ambulatory sites, mobile clinics, and physician office laboratories) during 1994-2005, based on previous Centers for Disease Control and Prevention studies. This poster presents the 2006-2016 PT performance trends of HI and AOT for analytes tested as a part of a CMP. **Methods:** Using the Centers for Medicare & Medicaid Services Quality Information Evaluation System (QIES) database, we analyzed PT scores from 2006 to 2016 and compared PT failure rates for the CMP analytes tested at HI and AOT. This equated to approximately 450,000 HI laboratory tests and 390,000 AOT laboratory tests. **Results:** Failure rates for the majority of analytes decreased steadily within the 10-year period for both HI and AOT. Notable exceptions where failure rates decreased for HI but increased for AOT within the last year of the 10-year period were observed for alanine transferase, alkaline phosphatase, bilirubin, creatinine, and glucose. **Conclusion:** The observed improvement in PT performance for both HI and AOT has important positive implications relevant to the impact of CLIA PT regulations. For both groups, failure rates within the last 10 years (2006-2016) are generally lower than the prior years (1994-2005). While contributing factors are not fully understood, it is likely that differences in laboratory staff retainment, testing methodologies (and equipment), and managerial factors (e.g. how staff is managed and supported) between HI and AOT may have contributed to the observed discrepancies in PT performance. Additionally, grading strategies of PT programs may also be a factor. PT helps clinical laboratories to ensure accurate patient test results; therefore, offering objective evidence of laboratory competence.

B-201

A Lean Project Study with Return on Investment (ROI) to Eliminate Defects Associated with False Positive Results on Urine Bilirubin Screening

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Background: Our medical center laboratory performs approximately 210 urine bilirubin screening tests each day as part of a urinalysis profile. Of these, approximately 50 percent have a positive urine bilirubin screening result (+1, +2, or +3) which are reported to our physicians and must be evaluated. Currently, physicians we interviewed estimate that follow-up on these results takes approximately 20 minutes (19 +/- 4.2 minutes). And of course, follow-up is required because a positive urine bilirubin result can indicate significant liver disease which, without proper treatment, can progress to severe morbidity and mortality. Defects noted in our bilirubin screening reveal that only 70 to 80 percent of +1 and +2 urine bilirubin results by our screening method actually reflect true positive results, thus making the false positive Type I alpha error rate essentially 75 percent. On the other hand, a +3 screening results has a much higher predictive value and hence a much lower false positive rate. Methods: To address this problem, we instituted a urine bilirubin confirmatory procedure involving 1) confirmation of all positive urine bilirubin screening results with a new IctoCheck method and 2) additional quantitative confirmatory testing on our Beckman Coulter DxC800 chemistry analyzer using both the diazo and enzymatic bilirubin assays. Results: Using these confirmatory tests we were able to reduce our false positive bilirubin urine screening defect rate from 75 percent to 3 percent. The total cost of the research and validation phase of the project including reagents, technologist's time, and faculty time was $1,546. The cost of providing the additional confirmatory assay was $884 per month. Conclusions: Our physician time savings was based on an average time savings of 19 minutes per case amounts to an estimated savings of $10,752 per month. The ongoing return on investment from this project based on physician time savings alone amounted to 1,216 percent. Valuable patient time is also saved and also the patient would avoid having a false positive result attached to their medical record. Moreover, this project has resulted in 1) reduction of the expense and risk of additional follow-up urine and blood testing for liver function assessment and 2) avoidance of the family and patient's concern regarding a potentially devastating disease involving the liver.

B-202

Comprehensive evaluation of the internal and external quality control to redefine analytical quality goals

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Background: The aim of this work is to design a selection algorithm for a Total Allowable Error (TEa) source using a graphic tool that, by integrating Internal (IQC) and External (EQC) Quality Control performances, enables the laboratory to evaluate which is the TEa source that better fits the test analytical performance. It is worth noting that in the model proposed by the laboratory, the selection of the highest-in-hierarchy TEa (according to the Milan 2014 Consensus) as possible was always a priority. **Methods:** The results of twelve surveys of the External Quality Evaluation Program EQAS® BIO-RAD from 2017 were used to evaluate the performance of twenty-tree biochemistry tests. The evaluated assays were processed in a homogenous system, Abbott manufactured analytical platform Architect ci8200 and reagent. Two analytical performance indicators (sigma metric and bias) were estimated for each test during the same period of time, the year 2017. Sigma metric was calculated through the results obtained in the IQC and the Bias was estimated based on the EQC program. The sigma metric was charted as a function of the bias expressed as the percentage of the TEa [Bias (%TEa)]. Following the proposed algorithm (considering the hierarchy in the Milan 2014 Consensus) the TEa was evaluated depending on 2 areas. One area in the chart was defined as the objective area ($5.15 < \text{Sigma} < 12$ and $\text{Bias } (\%TEa)_{2017} < 50$ or $\text{Sigma} > 12$ and $25 < \text{Bias } (\%TEa)_{2017} < 50$) in which the used TEa is the appropriate one for the analytical performance of the test under evaluation. For any test located outside said area (second area), a performance reevaluation was required using another source of TEa. **Results:** In 19 of the evaluated tests, their analytical performance allowed for the selection of biologic variability as TEa source. In the 4 remaining cases (Calcium, Magnesium, Total Protein and Sodium), State of the Art was selected. **Conclusion:** Jointly, the chart and the selection algorithm for TEa source, enabled the laboratory to standardize the selection procedure of the most appropriate TEa for the test analytical performance.

B-203

Overview of Laboratory Sanctions from 1996 to 2016

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Background: Every year the Centers for Medicare & Medicaid Services (CMS) publishes a list of laboratories that were found to have not been compliant with Clinical Laboratory Improvement Amendments of 1988 (CLIA). Objectives: To summarize and characterize the laboratories sanctioned by CMS or accrediting organizations from 1996 to 2016. Methods: Using the Sanctioned Laboratory Registry data, we compiled the laboratories recorded each year, summarized the types of sanctions and reasons, and linked the registry data with CLIA laboratory demographics to characterize the sanctioned laboratories. Results: Between 1996 and 2016, 3406 sanctions were recorded in the registry, representing 2924 individual laboratories and 100-250 sanctioned laboratories each year (Figure 1). The most common sanctions are certification revoke/suspense/limitation (1447, 35%), alternative sanctions (i.e., civil money penalty, 1328, 32%), and accreditation revoke/denial/withdrawn (1159, 28.3%). The most frequently cited reasons are proficiency testing failures (1589, 47%), out of compliance (861, 25%), deficiencies (491, 14%), failure to submit plans of corrections (316, 9%), and improper proficiency testing referral activity (232, 7%). The most common facility types of the sanctioned laboratories are physician office laboratories (1503, 51%), hospital laboratories (646, 22%) and independent laboratories (566, 19%). At the time of sanctions, most laboratories had a Certification of Accreditation (CoA, 1187, 44%) or a Certificate of Compliance (CoC, 1189, 44%), since only CoA and CoC laboratories are routinely surveyed. Among the 2924 sanctioned laboratories, 1495 or 51% had been deactivated by the end of year 2016, most of which (1107, 74%) were deactivated within 2 years of being sanctioned. Conclusions: As the most regulated aspect of laboratory practice, proficiency testing related issues are the most often cited reasons for sanctions. Deactivation appears to be the most common consequence of sanctions. Laboratory professionals, including laboratory directors, could use the lessons learned from the sanctions to improve practices, especially those related to testing quality.

B-204

Improving Intra-operative Parathyroid Hormone Analysis Turnaround Time

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Background: During parathyroidectomy, intra-operative parathyroid hormone (PTH) concentrations are used to guide surgeons in determining the extent of parathyroid excision. Delays in PTH analysis will lead to prolonged surgical time, increased cost, and increased risk of infection for patients. In February 2016, our institution implemented total lab automation in our core laboratory, including biochemistry analyzers. With this change, PTH analysis was moved from a point-of-care instrument in the operating room (OR) to the hospital's core laboratory. Turnaround times post change were unsatisfactory for surgical needs so an interdepartmental quality improvement project was initiated. **Objectives:** To shorten the transport time of each specimen from the OR to the laboratory to 10 minutes or less and shorten PTH analytical time to 20 minutes or less. **Methods:** The process for collecting, transporting, and analyzing an intra-operative PTH specimen was mapped in detail. Results showed that the most significant delays were in the delivery of the specimens to the laboratory and in longer than anticipated analytical time on the biochemistry automation. The root cause for the delay in transport time was attributed to the speed and unpredictability of the pneumatic tube system. In response, specimens were routed to the dumbwaiter currently used for surgical pathology specimens requiring frozen sections, which directly connects the OR to the laboratory for analysis. Delays in the PTH analytical time was attributed to the continuous stream of specimens entering the analyzer from the automation line, thereby interfering with the speed of analyzing the PTH specimens. This backup was remedied by requiring the OR nurse to notify the laboratory before a surgery started so that one of the automation lines could be stopped temporarily, allowing direct loading of the sample into the analyzer. **Results:** Median transport time of PTH specimens decreased from 12 mins to 6 mins by sending specimens through the frozen section dumbwaiter. Median analytical time decreased from 22 mins to 18 mins by stopping the automation line temporarily and manually loading the specimens.**Conclusion:** The median transport and analytical time of intra-operative PTH specimens was reduced from 34 mins to 24 mins (29%) by optimizing the workflow, resulting in better operative throughput and shorter surgery time.

B-205

Risk Analysis and Assessment Based on Sigma-metrics and Intended Use

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Background: To minimize the risk of harm to patients in the analytical process, a risk analysis and assessment model based on sigma-metrics and intended use was constructed, based on which differential Sigma performance expectations of 42 analytes were developed. **Methods:** Failure mode and effects analysis (FMEA) was applied to produce an analytic risk rating based on three factors, each test of which was graded as follows: 1) sigma-metrics; 2) the severity of harm; 3) intended use. By multiplying the score of sigma-metrics by the score of severity of harm by the score of intended use, each was assigned a typical risk priority number (RPN), with RPN≤25 rated as low risk. Low risk was defined as acceptable standards, the Sigma performance expectations were calculated in reverse. **Results:** Among the 42 analytes, tests with $\sigma \geq 6$, $5 \leq \sigma < 6$, $4 \leq \sigma < 5$, $3 \leq \sigma < 4$, $\sigma < 3$ were 21, 5, 5, 6, and 5, respectively; there were 7 high-risk tests, 8 of them medium risk tests. According to the risk assessment conclusion, 13 tests had Sigma performance expectations≥6; 15 test items had Sigma performance expectations≥5, while 3 test items had Sigma performance expectations≥4; 11 test items had Sigma performance expectations≥3. **Conclusion:** Using sigma-metrics and accounting for the intended use of test will help clinical laboratories design a comprehensive risk assessment system to identify the high-risk tests. The differential Sigma performance expectations can be also established by the RPN to satisfy the low risk to the patients and avoid repeated risk assessment.

The Risk Score of three novel factors			
Risk Score	Sigma-metrics	Severity	Intended uses
5	σ<3	Catastrophic	Diagnosis
4	3≤σ<4	Critical	Screening
3	4≤σ<5	Serious	Management
2	5≤σ<6	Minor	-
1	σ ≥6	Negligible	-

B-206

Evaluation of Positive Frequency as a Quality Indicator for Assay Performance

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Background: Quality assurance and quality control procedures in the clinical laboratory ensure that the patients' results are reported with sufficient accuracy and reproducibility for clinical use. Quality control (QC) material is used to detect random assay error and systemic assay bias as well as long term shifts and trends. However, QC material may be susceptible to matrix effects which may cause it to behave differently from patient specimens. For some clinical tests, the frequency of distribution of patient results provides a potentially useful quality assurance indicator. In theory, the percent of patient results with values above the reference interval (positive frequency rate), may be altered by changes in patient population, but also can potentially provide insight into analytical shifts over time. Currently, the effects that changes in assay performance might have on positive frequency rates are not well understood. **Objective:** This study compares shifts in positive frequency rates with QC trends for several widely utilized markers to determine if there is a correlation between these two quality indicators within a large clinical reference laboratory. **Methods:** Positive frequency rates and quality control (QC) values were simultaneously tracked for several markers on Beckman Coulter Unicel DxI 800 immunoassay. Monthly QC and positive rates for alpha-fetoprotein (AFP), inhibin A (INHA), cancer antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), and thyroglobulin (Tg) were recorded from January 2016 to September 2017. The number of tests per month on average was 2200 for AFP, 400 for INHA, 2200 for CA19-9, 4500 for Tg, 1000 for CEA and 1200 for FT3. For free triiodothyronine (FT3) data was recorded from January 2015 to October 2016. Observed monthly shifts in positive frequency were plotted relative to shift in monthly mean QC values for the controls that were closest to the positive/negative decision limit. **Results:** The coefficient of variation (CV) of positive frequency rates over this period for AFP, CA19-9, CEA, INHA, FT3 and TG was 5.3 %, 2.4%, 3.7%, 10.1%, 56.0% and 3.9%, respectively. The percent CV for QC values for AFP, CA19-9, CEA, INHA, FT3 and TG were 2.9%, 5.3%, 14.2%, 2.6%, 5.3% and 13.8%, re-

spectively. Correlation plots showed no correlation between monthly QC value and positive frequency shifts. However, FT3 provided an example where a significant change in the monthly positive rate reflected a change in assay performance that was not detected by QC. A reagent lot change resulted in a ~20% increase for the positive frequency with only a +0.8% shift in corresponding monthly QC values between February and April 2016. Further investigation revealed a reagent formulation change by the manufacturer that had resulted in a potential 10-14% increase in patient values across the reference range that was not reflected in QC data. **Conclusion:** Positive frequency was evaluated as a potential quality metric for assay performance. Our data suggests that positive frequency shifts are independent of monthly QC value shifts. However, in certain circumstances, monitoring frequency shifts can enable detection of assay changes and suggests that positive frequency monitoring has value as an additional quality indicator.

B-207

Consolidation of Therapeutic Drug Monitoring (TDM) Testing to the Automated Core Lab and Validation of Serum Separator Tubes (SST) for Drug Testing Yields Improved Turn Around Time (TAT) and Reduces the Number of Blood Tubes Collected

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Background: With improved technology and demand for faster results, clinical laboratories have an obligation to continuously reevaluate the processes for obtaining samples, testing, and delivering results to physicians. To improve efficiency, 23 therapeutic drug monitoring (TDM) tests were transferred from the specialty toxicology laboratory to the automated core laboratory. Process improvement opportunities were identified including validating automation-ready serum separator tubes (SST, Becton-Dickinson) to replace traditional red top serum tubes for 14 of the drugs. The expectation was that efficiency and turn-around-time (TAT) would be improved by consolidating testing to the core lab. Two tests were chosen as surrogates to measure the TAT improvement. **Objectives:** The aims of this study were to: 1) Assess TAT for phenytoin before and after consolidation of TDM testing from the specialty toxicology laboratory to the core laboratory; 2) Assess TAT for phenobarbital before and after consolidation in conjunction with a specimen type change from red top tube to SST; and 3) Evaluate whether changing the specimen type to SST for phenobarbital reduced the number of blood tubes collected when phenobarbital was ordered. **Methods:** Physician orders for phenytoin and phenobarbital were extracted from the laboratory information system (LIS) for one year pre-consolidation (2014) and one year post-consolidation (2017). Inpatient and outpatient specimens collected on-site (Mayo Clinic Rochester hospitals or clinics) were included. TAT was calculated as time (minutes) from blood collection to result verification. The mean +/- standard deviation (SD) TAT and % of orders meeting a TAT goal of 140 minute for phenytoin (red top tube) or 120 minutes for phenobarbital (SST) were calculated. To determine whether the change to SST impacted the number of blood tubes collected per patient when phenobarbital was ordered, post-consolidation phenobarbital orders were reviewed to determine whether other tests were ordered concurrently. **Results:** The TAT for phenytoin was 147 ± 56 minutes (n=220 orders) prior to consolidation (2014) and 90 ± 38 minutes (n=532 orders) post-consolidation (2017). The % of orders meeting the 140 minutes TAT goal was 42% pre-consolidation and 92% post-consolidation. The TAT for phenobarbital was 162 ± 78 minutes (n=311 orders) prior to consolidation (2014) and 74 ± 35 minutes (n=251 orders) post-consolidation (2017). The % of orders meeting the 120 minute TAT goal was 29% pre-consolidation and 94% post-consolidation. In 2017, 220 of the phenobarbital orders had additional laboratory tests ordered concurrently. The change to an SST for phenobarbital eliminated an additional blood collection tube in 60 cases (27%) and initiated an additional tube in 35 cases (16%). **Conclusions:** Using phenytoin and phenobarbital as representative tests, our results demonstrate that consolidating TDM testing from a separate specialty laboratory to the automated core lab greatly improved TAT. In addition, by changing from a red top tube to SST there was a reduction in the number of blood tubes collected when phenobarbital was ordered. This suggests that specimen type consolidation, when properly validated, is another mechanism by which laboratories can improve efficiency.

B-208

Derivation of short term biologic variation of platelets in inpatients with thrombocytopenia

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Background: Biologic variation (BV) has become the primary criterion for deriving specifications for maximum allowable analytic error. While BV is usually assessed in healthy subjects, (generally with strict observation of preanalytical conditions), it is not clear whether similarly derived BVs are applicable in subjects with abnormal concentrations of the analyte in question. We use a unique methodology (Cembrowski et al, Clin Chem Lab Med) to derive BV from consecutive intra-patient data and demonstrate that short term platelet BV is significantly higher than the usual 3%. **Methods:** A data repository provided all of the low platelet results measured over a 2 year period at the University of Alberta and Royal Alexandra Hospitals in Edmonton, Canada). These measurements were made on tandem Sysmex XN 9000 analyzers. A total of 14,000 platelet count pairs under 120,000 were collected and were associated with both normal hemoglobin and neutrophil counts. We tabulated the pairs of sequential intra-patient platelet tests that were separated by 0-4, 4-8, 8-12,⋯ up to 48 hour intervals. The standard deviation of duplicates (SDD) of the paired platelet determinations was calculated for each time interval. The graphs of SDD vs. time interval were linear; the y intercept provided by the linear regression equation represents the sum of the BV and short term analytic variation (s_a): $y_0=(sa^2 + BV^2)^{½}$. s_a was determined from onsite control analysis. **Results:** The Table summarizes the ST platelet BV for 4 different platelet ranges. **Conclusions:** The relative platelet imprecision for patients with thrombocytopenia is about twice that of normal individuals (3.2% from Buoro et al, Clin Chim Acta 470 (2017), 125-132). As such, evaluations of platelet measurements in the thrombocytopenic range might permit higher allowable error. The y intercept multiplied by 2.5 yields 15%. Platelet increases or decreases of 15% in thrombocytopenic patients are statistically significant (P<0.01).

Patient Platelet Range x10⁹/L	Median Platelets x10⁹/L	s_a, x10⁹/L	y_0 x10⁹/L	Short term BV x10⁹/L	Relative Short term BV (%)
<120	95	7.76	5.5	5.5	5.8%
<100	79	7.24	5.4	4.9	6.2%
<80	60	6.38	5.2	3.7	6.1%
<60	43	5.77	5.1	2.7	6.2%

B-209

Establishment of Analytical Performance Goals Based on Total Error of Patient Misclassification

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Background: The concept of total allowable error (TEa) was initially introduced to assess analytical performance of laboratory assays. A limitation of TEa is that its quantitative relationship to patient test misclassification is unclear. The objective of this study was to develop a new analytical performance goal based on minimizing test misclassifications. **Methods:** Simulation analysis was performed on 14 CLIA-regulated analytes to assess the impact of imprecision and systematic bias on total error of misclassification. For each analyte, 230,000 measurements were obtained with the Dimension Vista® system, between October 2008 and July 2012 at the National Institutes of Health. CLIA TEa limits were used for the analysis and the manufacturer's recommended upper and lower reference range were used to classify subjects. **Results:** No bimodal distributions were observed for any of the tests. Instead, distributions containing "heavy tails" were observed; therefore, we developed a mixed single population model and introduced either fixed bias or imprecision by simulation, with either a single or a double cutpoint. We observed a complex relationship between bias and imprecision, but for relatively low errors they had a similar impact on misclassification. As seen in the example of glucose (Figure 1), a TEa limit of 10% corresponds to 15% of misclassification for positive bias, and 20% for negative bias. Using this approach, we developed new equations for each analyte that allow laboratories to stay within the CLIA TEa limits and calculate what the limit represents in terms of patient misclassification. **Conclusion:** Our new index based on test misclassification has the

advantage that it correctly takes into account the differential effect of bias and imprecision. It provides a more intuitive number for assessing both analytical performance goals and the clinical impact of test errors.

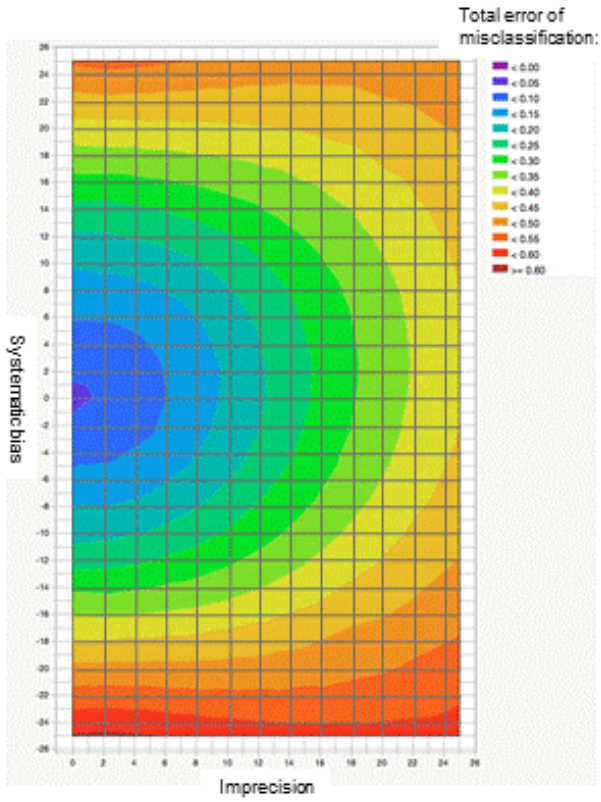

Figure 1: Impact of bias and imprecision on total error of misclassification (TEM) for glucose. The correlation between imprecision/bias and TEM is $\frac{imprecison^2}{X-intercept^2}+\frac{bias^2}{TEa^2}=1$. X-intercept, intercept of TEM contour line with X axis where the y axis is zero.

B-210

PCT-guided antibiotic stewardship versus usual care for hospitalised patients with suspected sepsis or lower respiratory tract infections in the US: a model-based cost analysis

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Background: Procalcitonin (PCT) is a biomarker that supports clinical decision-making regarding initiation and discontinuation of antibiotic therapy. It is demonstrated to be safe and effective in identifying patients who do not require initiation of antibiotic therapy and in reducing antibiotic duration, thereby supporting global efforts to reduce unnecessary antibiotic utilization. Several cost(-effectiveness) analyses have been conducted on PCT-guided antibiotic stewardship, but so far none used US originated data.**Objective:** To compare effectiveness and costs of a PCT-algorithm to guide antibiotic prescription versus standard care for patients hospitalized with a diagnosis of suspected sepsis or lower respiratory tract infection (LRTI) in the US. **Methods:** A previously published health economic decision model was used to compare the costs and effects of PCT-guided care. The analysis considered the societal and hospital perspective with a time horizon covering the length of the hospital stay. The main outcomes were total costs per patient, including treatment costs and productivity losses, the number of patients with antibiotic resistance or *C.difficile* infections, and costs per antibiotic day avoided. Multiple sensitivity and scenario analyses were performed to test the robustness of model outcomes. **Results**: PCT-guided care for hospitalized patients with suspected sepsis and LRTI is associated with a reduction in antibiotic days, a shorter length of stay on the regular ward and the ICU, shorter duration of mechanical ventilation, and fewer patients at risk for antibiotic resistance or *C.difficile* infection. Total costs savings in the PCT-group compared to standard care were estimated to range between 20%-30% for patients with suspected sepsis and LRTI, depending on specific data inputs and scenarios modeled. Projected reductions in number of patients with ABR were approximately 5% to 23% and reductions in *C.difficile* infection ranged from circa 55% to 63%. Cost-savings were mainly driven by the reduction in LOS and a shorter duration of mechanical ventilation. **Conclusion:** Using a PCT algorithm to guide antibiotic use in sepsis and hospitalized LRTI patients is expected to generate cost-savings to the hospital that are sufficiently large to recoup the upfront costs of PCT-testing and lower the rates of antibiotic resistance and *C.difficile* infections in populations of patients hospitalized with suspected sepsis or LRTI.

Wednesday, August 1, 2018

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

Mass Spectrometry Applications

B-211

Determination of Methylmalonic acid in urine by LC-MS/MS.

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Methylmalonic acid (MMA) is a specific diagnostic marker for vitamin B12 deficiency and for the methylmalonic acidemia. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization is a rapid, robust and selective technique. So the objective of this work was to develop and validate a simple method for the determination of methylmalonic acid in urine by LCMS/MS for application in clinical diagnostic. Chromatographic separation was obtained on Waters Acquity UPLC BEH C18 column (50 mm x 2.1 mm x 1.7 µm) held at 30°C using isocratic mobile phase constituted by 45% of water, 45% of acetonitrile, 10% of methanol and 0.1% of formic acid at a flow rate of 0.400 mL min^{-1}.The chromatographic run time was 4.10 min. The experiments were performed on an Agilent 6460C (Santa Clara, CA) triple quadrupole LC-MS/MS system operated in a positive mode, with an Agilent 1290 Infinity LC system. For the sample preparation, 100 µl of sample was spiked with 20 µl of deuterated internal standard and 30 µl of Hydrochloric acid 0.86 $molL^{-1}$ solution. The mixture was stirring for 5 seconds, 500 µL of ethyl acetate was added and it was vortexed for more 90 s. This mixture was centrifuged and 350 µL of the supernatant was evaporated to dryness with a vacuum concentrator. The extract was derivatized with 100 µl of n-butanol in hydrochloric acid 3.0 $mol.L^{-1}$. Linearity was achieved from 0.2 to 98.2 $\mu mol.L^{-1}$. The average of recovery was 94.1-110.1%. The intra and inter day imprecision was lower than 10.3%. In conclusion, the method has been developed and validated successfully for the quantitative analysis of MMA in urine and can be applied in clinical diagnosis.

B-212

Selective, simple and fast determination of Pyridoxal 5-Phosphate in plasma samples using a C8 column and LC-MS/MS analysis.

B. F. P. Paulo, E. Cueva Mateo, A. C. S. Ferreira. *Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil*

Vitamin B6 is important for normal brain development and for keeping the nervous system, immune system and skin healthy. Vitamin B6 has several derivatives, where pyridoxal 5-phosphate (PLP) has been determined to be the biologically active form due to its role as a cofactor in a number of enzymatic reactions. Patients with kidney disease or deficiency in absorption of nutrients are able to be vitamin B6 deficient. PLP is an acid and very polar compound (logP -2.09) and their chromatographic separation is difficult due to its poor retention in C18 reversed-phase (RP) columns. Although the MS/MS is considered a technique with excellent selectivity, we was observed that there an interfering peak close to the PLP retention time when a C18 RP column are used (Zorbax Eclipse Plus C18 RRHD 100 mm X 2.1 mm X 1.8 µm - Agilent Technologies). This interfering peak compromises the exact quantification of PLP and are present in 2-3% of samples analyzed in our laboratory. To solve this problem, was developed and validated a selective and simple analytical method for determination PLP in plasma by LCMS/MS using a C8 RP column. C8 columns are more polar than C18 columns and is capable to retain better the PLP to provide adequate chromatographic selectivity. The chromatographic separation was performed on Agilent Poroshell 120 HPH C8 column (50 mm X 3.0 mm X 2.7 µm) held at 25 °C using a gradient separation constituted of mobile phase A - 0.1% of acid formic solution and B - methanol with 0.1% of acid formic, at a flow rate of 0.400 mL min^{1}. The chromatographic run time was 3.2 min. All experiments were performed on Agilent 6460C (Santa Clara, CA) triple quadrupole LC-MS/MS system, with an Agilent 1290 Infinity LC system. The source was operated in positive mode. Sample preparation was performed adding 40.0 µL of plasma sample, calibrator or quality control and 130.0 µL of 5% trichloroacetic acid solution with 258.0 ng mL^{-1} of internal standard (PLPD3) to conical bottom PCR plate. The plate was sealed and vigorously agitated for 60 seconds and then, centrifuged at 4.500 rpm for 10 minutes. The supernatant was collected and injected on a LC-MS/MS system. The method was successfully validated achieving a LoD of 0.5 ng mL^{-1}, linearity of 3.0 to 120.0 ng mL^{-1} and imprecision was less than 5.3%. The recovery was between 95.5 and 85.3%. The selectivity was tested by analysis of 427 real samples. No interfering peaks was detected near the PLP retention time. In conclusion, the method has been developed and validated successfully for the quantitative analysis of PLP in plasma samples.

B-213

A rapid LC-ESI-MS/MS method for quantification of plasma oxysterols with dimethylglycine derivatization

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Background: Oxysterols are derivatives of cholesterol which are formed by oxidation through numerous chemical reactions and play an important role in many physiological processes and in various degenerative and metabolic diseases, lipid metabolism disorders. In this study, analytical validation of liquid chromatography-tandem mass spectrometric (LC-MS / MS) method was evaluated by using various extraction and derivation steps in the measurement of C-triol and 7-KC which are important oxysterols. **Methods:** Optimization studies were performed in LC-MS / MS with positive electrospray ionization (ESI) in multiple-reaction monitoring (MRM) mode. The analytical validation of C-Triol and 7-KC multiplex measurements with dimethylglycine (DMG) derivatization was evaluated in plasma from 25 healthy individuals. For this purpose; linearity, accuracy, repeatability, detection and quantitation limits (LLOD and LLOQ), recovery and carry-over analysis were studied and the results were evaluated statistically. **Results:** The time of the analysis was 10 minutes (min) for both parameters (C-Triol: 3.2 min, 7-KC: 7.4 min). The values of r-squared (r^2) were found for C-triol as 0,999, for 7-KC as 0,994 in generated calibration curves. Concentrations of C-triol and 7-KC were within ± 20% in quality control samples prepared at least two levels for accuracy analysis. Plasma C-triol levels were determined in a control group of 25 individuals (min: 15.7, max: 38.61; mean±SD: 25.61±9.2 ng/mL). The standard deviations were found high for 7-KC measurements, it was considered that the plasma matrix interfered or the 7-KC stability in the plasma deteriorated. **Conclusion:** In this study, oxysterol measurement has been developed and ready for use in our laboratory with a sensitive and specific method. 7-KC was considered to be a useful parameter to reflect the stability of the analyte in the plasma.

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Rapid preparation for analysis of steroid hormones by LC-MS/MS with Microelution at positive pressure.

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Background: Steroid hormones are of great importance in clinical endocrinology. However, the immunoassay method analyzes have methodological interferences that, in the majority, result in false high results. Liquid chromatography tandem mass spectrometry (LC/MS/MS) is replacing classical methods for steroid hormone analysis. It offers analytical specificity superior to that of immunoassays or conventional high performance/pressure liquid chromatography (HPLC) for low molecular weight analytes. However, the preparation time of the samples in the LC-MSMS may be a limiting factor for the analysis of a large number of samples. **Objective:** This study evaluates the reduction of preparation time of the samples through the process of positive pressure extraction with solid phase extraction cartridge and subsequent analysis for LC-MS/MS composed of an ACQUITY UPLC system combined with a XEVO tandem quadrupole mass spectrometer both from Waters. It was controlled by the MassLynx software **Methods:** We analyzed Testosterone, Androstenedione, 17α-hydroxyprogesterone, 11-deoxycortisol, Progesterone, Corticosterone, DHEA and cortisol, serum samples were prepared in the same extraction method and in only a single chromatographic run. All with deuterated steroid standards and solvents were purchased from SIGMA, Cabridge Isotopes Laboratories (CIL) and Merck. Samples were extracted using the positive pressure-96 Waters and the Oasis Prime HLB microelution cartridge. The LCMS system used was Waters ACQUITY UPLC, Xevo TQ-S positive ionization mode (ESI +) with ACQUITY UPLC BEH C18 2.1x100mm, 1.7µm column. The flow rate was 0.5mL / min. with gradient of solvents A and B, consisting of water and methanol with 0.1% formic acid respectively. Running time is 3.5 minutes. **Results:** To validate the method, we performed intra-day and inter-day comparative studies, for a total of 40 samples for each analyte. The method exhibited linear response in the range of 0.2 ng / mL to 250 ng / mL and CVs within the expected range. **Conclusion:** The use of micro-elution cartridge in positive pressure equipment has reduced the number of steps, eliminating evaporation and reconstitution processes, and optimizing sample preparation compared to traditional cartridges.

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Development and validation of Nicotinamide in human serum by liquid chromatography electrospray ionization tandem mass spectrometry.

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Nicotinamide (NA) is the active form of vitamin B3 and is an essential component of several coenzymes. A rapid LC-MS/MS method was developed for quantitative determination of nicotinamide in human serum 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* 122.9→80.0 for NA quantifier, 122.9→78.0 for NA qualifier and 128.9→85.0 for internal standard NA-$^{13}C_6$. Chromatographic separation was performed on a Poroshell 120 PFP column (100 mm x 2.1 mm, 2.7 µm) held at 30°C and isocratic mobile phase containing water:methanol (95:5, v/v) with 0.1% de formic acid at a flow rate of 0.2 mL.min^{-1}. The chromatographic run time obtained was 2.8 minutes. The extraction procedure is a simple protein precipitation with 400 µL of trichloroacetic acid 10%, using only 100 µL of sample and 25 µL of internal standard (NA-$^{13}C_6$). The linear range was achieved from 5.0 to 2000.0 µg.L^{-1}. The medium range of recovery was between 91 and 102%. Total imprecision ranged from 1.2 to 5.1%. The tests of quantification limits, linearity, precision and recovery were adequate for clinical evaluation. In conclusion, a simple and rapid method has been developed successfully for the quantitative analysis of vitamin B3 in human serum for clinical diagnosis.

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LC-MS/MS assay for detection of elevated biotin in plasma

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BACKGROUND: Elevated biotin due to self-administration or prescription is a potential interferent for many immunoassays that utilize biotin-streptavadin interaction in their design. A recent FDA Safety Alert (November 2017) has brought widespread current attention to this issue. Laboratories will ideally be prepared to assess interference in individual cases by pre-treatment of samples with streptavadin. A biotin assay can assist in validation of such procedures, as well as provide a means for direct verification of biotin elevation in suspected cases. To this end, we developed a simple LC-MS/MS assay designed specifically to measure elevated biotin in plasma (biotin >1 ng/mL). **METHODS**: Stock solution (1000 ng/uL) of biotin (Sigma-Aldrich) was used to prepare elevated biotin standards (0, 1, 20, 100, 500 ng/mL) by addition to 7% bovine serum albumin (BSA). Desthiobiotin (Santa Cruz Biotechnology) was used as internal standard (IS). Samples were prepared as follows: 200 ul of MeOH containing IS was added to 200 uL of sample; after vortexing and centrifugation, 100 uL of supernatant was diluted with 50 uL of mobile phase A (H_2O, 0.1% formic acid). 20 uL of the final extract was injected for LC-MS/MS. LC (Shimadzu UFL Prominence) was performed using a Phenomemex Synergy Hydro RP column (50 × 4.6 mm) at 50 oC, with binary mobile phase (B = MeOH, 0.1% formic acid) at fixed flow rate of 0.6 mL/min as follows (interval (min), %B): -1-0, 30%; 0-3.5, 30%-80%; 3.5-4.5, 80%; 4.5-5.0, 30%. MS/MS was performed using positive APCI on a Sciex 3200MD and monitoring for m/z 245.1>227.1 (biotin) and m/z 215.2>179.1 (IS). Retention times were 2.4 min (biotin) and 3.1 min (IS), with injection-to-injection time of 6.0 min. Quality control samples were prepared in 7% BSA from biotin purchased from Santa Cruz Biotechnology. **RESULTS**: The assay was linear over the range of standards (r2>0.99, 0-500 ng/mL). Accuracy was verified by 1:1 ± 4% correspondence with biotin standards from an independent source (Vector Laboratories). Process recovery was 51% at 100 ng/mL biotin. The lower limit of quantification (LOQ) in plasma was 2 ng/mL (CV=8.4%), which is greater than the upper limit of the reference range for biotin (nominally 1 ng/mL). Intra- and inter-assay coefficients of variation were <3.9% and <4.3% at 10 and 100 ng/mL, respectively. No interferences were found in measurements made using biotin-spiked high QC materials (Biorad) for a wide range of immunoassay analytes and drugs of abuse, or for samples with elevated hemoglobin, hyperbilirubinemia or lipemia. Matrix effects, if any, were stable and equal across standards, controls and patient samples as assessed by metric plots for IS. Pooled plasma demonstrated biotin below LOQ. A survey of 20 individual patient plasma samples showed no results above LOQ. **CONCLUSIONS**: The LC-MS/MS assay showed acceptable performance characteristics for quantitation of plasma biotin elevated beyond the upper limit of the reference range. Availability of such an assay will be useful in validation of biotin-stripping procedures used to remove immunoassay interference, as well as for direct evaluation for elevated biotin in suspected interference case samples.

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LC-MS/MS method for detecting paclitaxel in human plasma

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Background: Paclitaxel is a natural product commonly used to treat a wide range of cancers including ovarian, breast, lung, bladder, prostate, melanoma, esophageal, and Kaposi's sarcoma. Recently, exposure-guided paclitaxel dosing (i.e. therapeutic drug monitoring - TDM) was demonstrated to be less toxic and similarly effective to standard dosing when combined with platinum drugs in patients with lung cancer. As efforts continue to develop new drugs to treat the sequelae of chemotherapy or to enhance the efficacy of paclitaxel, it will be important to confirm that its pharmacokinetics are unaltered and anti-neoplastic efficacy is maintained. Therefore, TDM of paclitaxel and/or monitoring the possible interaction of paclitaxel with other drugs is worth further investigation in additional tumor types and chemotherapy regimens. To this end, we developed a rapid and sensitive LC-MS/MS method for measuring paclitaxel in human plasma. **Methods:** Human plasma samples were treated with stable isotope labeled paclitaxel-d5 solution in methanol to precipitate proteins and analyzed by ABSciex 5500 Triple Quadrapole mass spectrometer with electrospray ionization in a positive ion mode. A Shimadzu Nexeral LC equipped with a Phenomenex C18 column (50 x 3.0 mm, 2.6 µm) and a Phenomenex C18 guard cartridge (4.0 x 2.0 mm) was used for the analyte separation. Mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Starting mobile phase consisted of 20% B at 0.7mL/min flow rate, transitioned to 60% B from 0.3 to 0.6 min, 100% B at 0.75 min, and reverted to 20% B at 4.6 min. For sample preparation, 100 µL of matrix blank, calibrators, controls, and samples were mixed with 300 µL of internal standard solution and centrifuged at 16,500 g for 5 min. Then 250 µL of each supernatant was mixed well with 250 µL water. 25 µL of each prepared sample was analyzed by LC-MS/MS. The results were quantified using the internal standard method of quantitation. **Results:** The developed method was specific for paclitaxel with no interference of peak at the retention time of paclitaxel. Linearity (r^2 >0.99) was established for the range of 10-1000 ng/mL. Coefficients of variation for low, middle, and high quality controls were less than 4.6% for intra-day assays and less than 7.2% for inter-day assay. Accuracies for all quality controls were greater than 94.5% for both intra- and inter-day assays. Further validation studies did not show apparent matrix effects (2.3%) and carry-over (-2.2%). **Conclusions:** The developed LC-MS/MS method can be used to quantify blood paclitaxel concentrations in cancer patients during chemotherapy with minimal sample preparation, rapid turn-around, and acceptable precision and accuracy.

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Validation of a LC-MSMS method for the quantification of plasma curcumin levels in clinical analyses

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Background: Curcumin is a polyphenol with a long history of being used as a dietary spice with a broad spectrum of effects described in the medical literature as antioxidant, anti-inflammatory, antimicrobial and anticancer. The objective of this study was to develop and validate a method with sensitivity and specificity enough to be used in clinical studies with curcumin. **Methods:** Standards for Curcumin and Curcumin-D6 were acquired from Sigma-Aldrich and Toronto Research Chemicals, respectively. A Waters Corporation Acquity TQD was used in positive ionization mode with an electrospray source. Samples were collected in plasma, and the calibrators were made by adding the standards in blank plasma. Samples and calibrators were extracted by addition of zinc sulfate 0.1M and acetronitrile followed by centrifugation and separation of the supernatant. The triple quadrupole mass detections with multiple reaction monitoring mode was used to monitor the ion transitions (m/z) 369.1>177 and 369.1>285 for curcumin, and 375>180 for the internal standard curcumin-D6. The method was validated following the CLSI C62-A document. After validation the pharmacokinetics was tested in a healthy volunteer by drinking a curcumin mixture with 200 mg of curcumin. **Results:** The lower limit of detection was 0.13 ng/mL, the lower limit of quantification was 2.3 ng/mL, and the linearity was 1021.0 ng/mL. The intra-day and inter-day precision was calculated on two levels. The intra-day CV was 11.6% for the low level and

6.8% for the high level. The inter-day precision was 6.5% for the low level and 4.9% for the high level. Recovery was determined on three levels and the results were 99.0 to 104.6%. The matrix effect was calculated in three levels by addition of curcumin standards in blank plasma compared to the standard in mobile phase. The results were 0.60, 1.2, and 2.3%. The method was tested for carry-over and none was noticed at the highest tested concentration (1200 ng/mL). The sample was shown to be stable for 15 days in a -30°C freezer. For the pharmacokinetics the maximum concentration (C_{max}) was 236.6 ng/mL and the time to achieve maximum concentration (T_{max}) was 90 minutes. **Conclusion:** The method was shown to be simple, fast, and reproductible. This provides an analytical tool to study the pharmacokinetics of curcumin and on clinical studies with curcumin

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Measurement of Thiopurine Metabolites in Erythrocytes to Optimize Thiopurine Therapy

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Background: Azathioprine (AZA), 6-Thioguanine (6-TG) and 6-mercaptopurine (6-MP) are thiopurine-based molecules requiring enzymatic conversion into thiopurine nucleotides to exert cytotoxicity for the treatment of acute lymphoblastic and myeloblastic leukemia. The metabolism of AZA and 6-MP by a series of enzymes including Thiopurine Methyltransferase (TPMT) yields 6-thioguanine nucleotides (6-TGN) whose levels correlate with therapeutic efficacy and 6-methylmercaptopurine (6-MMP) nucleotides which are associated with hepatotoxicity and myelotoxicity. TPMT activity is affected by genetic polymorphisms with significantly different activity profiles. Hence, the accurate quantification of thiopurine metabolites is clinically informative and essential in the mitigation of toxicity. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with multiple reaction monitoring (MRM) was developed by the Clinical Pharmacokinetics Laboratory at St. Jude Children's Research Hospital to assay for 6-TGN and 6-methylmercaptopurine (6-MMP) nucleotides in erythrocytes. The aim of the current study was to transfer the assay to an LC-MS instrument platform at the Advanced Clinical Chemistry Diagnostics Laboratory at Johns Hopkins, harmonize the assays between platforms from different LC-MS vendors, and to validate the assay towards a goal of offering the assay for clinical testing purposes. **Methods:** Remnant, de-identified whole blood specimens collected in K_2-EDTA tubes were used to prepare 6-TG and 6-MMP calibrators and QC samples in lysed erythrocytes. 6-TG-$^{13}C_2$,^{15}N and 6-MMP-d_3 (Toronto Chemicals) were used as internal standards (IS). Red blood cells (RBC) were counted using a Sysmex XP cell counter. 6-TGN and 6-MMP nucleotides extracted from erythrocytes were converted to their respective bases by acid hydrolysis. Reversed phase HPLC was conducted with a Vanquish system (Thermo Scientific). A 2.8 min binary gradient (6 min total run time) from 2 - 80% mobile phase B (0.1% formic acid in acetonitrile) was delivered to a 2.1 x 50mm high strength silica column (Waters). The column was interfaced with an Endura triple quadrupole mass spectrometer (Thermo Scientific). A constant dwell time of 10 ms was used and two transitions were monitored for each analyte. Results were reported in units of pmol/8x10^8 RBCs. Validation experiments included linearity, LLOQ, precision/repeatability, accuracy, specificity and robustness. The clinical performance of the assay was evaluated by measuring 6-TGN and 6-MMP nucleotide levels in acute lymphoblastic leukemia patients who received thiopurine therapy at St. Jude Children's Research Hospital. **Results:** The thiopurine metabolites assay was successfully transferred and adapted to an LC-MS platform distinct from the instrumentation that was used to develop the original method. Calibrators from St. Jude Children's Research Hospital were used to enable instrument method optimization towards a goal of inter-laboratory harmonization. Additional studies are ongoing to explore the feasibility of coupling this LC-MS based test with a TPMT genotype test to enable the prediction of patient response by assessing TPMT activity while providing clinicians with clinically actionable data to facilitate the optimization of drug concentrations by measuring thiopurine drug metabolites. **Conclusion:** An LC-MS thiopurine metabolites assay was validated and harmonized across distinct liquid chromatography and mass spectrometry instrument platforms to permit the accurate determination of thiopurine metabolite concentrations to optimize drug dosing and prevent toxicity in leukemia patients.

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Rapid determination of 25-hydroxyvitamin D by supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS)

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Background: Determination of 25-hydroxyvitamin D has become a critical test for evaluation of calcium metabolism and homeostasis. At present, liquid chromatography-mass spectrometry (LC-MS) is the most reliable method. However, 25-hydroxyvitamin D could not produce a strong signal in LC-MS due to its lack of positive charge. To ensure the detection of 25-hydroxyvitamin D in low concentration, the system noise has to be strictly controled by using expensive ultra pure solvent as mobile phase. In this study, a supercritical fluid chromatography-tandem mass spectrometry (SFC-MS/MS) method was developed for determination of 25-hydroxyvitamin D. Inert, non-toxic, non-flammable, and low cost supercritical carbon dioxide was applied as eluent with modification of methanol in small amount. 25-hydroxyvitamin D was analyzed within less than four minutes. **Methods:** The separation was performed using a SFC system coupled to a triple quadrupole mass system. The backpressure was set by the automatic back pressure regulator. Human plasma samples were extracted using a liquid-liquid extraction method. Calibration curves, limit of detections (LODs), intra-assay precision, and accuracy were calculated for method validation. **Results:** The 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 were baseline separated. The calibration curves were linear from 1 to 200 ng/mL with regression coefficients (R^2) > 0.99. The limits of detection (LOD) were found to range between 0.5 and 5 ng/mL for 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3. The intraassay relative standard deviation (RSD) was lower than 10%, and the accuracy of QC sample was between 92% to 110%. **Conclusion:** A new SFC-MS method was developed for determination of 25-hydroxyvitamin D within four minutes. A baseline separation of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 with satisfied precision and accuracy was achieved by optimizing the outlet pressure, the column temperature, the flow rate and the methanol gradient program.

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Vitamin D and its metabolites- Which LC-MS methodology do I choose?

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Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC-MS/MS) is suited for rapid analysis of multiple analytes. Various LC-MS/MS analytical methodologies were developed for the quantitation of the Vitamin D metabolites such as 25-Hydroxy-Vitamin D, 1,25-Dihydroxy-Vitamin D, 24,25-Dihydroxy-Vitamin D and others to ascertain under which LC and MS conditions and sample preparation options resulted in the most consistent and appropriate results. A simple sample preparation technique that involved a simple protein crash and liquid-liquid extraction were utilized along with a one (1D) dimensional liquid chromatographic configuration with and without the PTAD derivatization. The described method achieves the required sensitivity and is capable of determining the various vitamin d metabolites over their dynamic range. Therefore, simple and accurate quantitative analytical methods were developed for the quantitatively measurement of vitamin D metabolites in serum. Method: A Thermo Scientific™ Quantis™ tandem mass spectrometer in positive Electrospray and Atmospheric Pressure Chemical Ionization modes and a Thermo Scientific™ Dionex™ Vanquish™ Horizon HPLC system were initially utilized for this analysis. 200 µl of serum were used for the analysis of the various vitamin D metabolites in serum. Various columns were evaluated and a Thermo Scientific™ Accucore™ C18 100 x 2.1 mm, 1.5 µm with a water:methanol mixture containing and 0.1% Formic Acid along with methylamine achieved baseline chromatographic separation for all the vitamins D metabolites in serum in less than 8 minute run time. Quantitative analysis was performed using selective reaction monitoring (SRM) with transition pairs for each analyte and internal standard in positive mode and the precision and accuracy of the method was verified using pooled quality control materials and serum samples. Result: Good linearity and reproducibility were obtained with the concentration range of 5 pg/ml to 1000 ng/ml for the respective vitamin D metabolites in serum with a coefficient of determination >0.95 for the sample preparation technique and the LC-MS/MS configuration used. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 5 pg/ml to 50 pg/ml and excellent reproducibility was observed for all compounds (CV < 10%). Conclusion: Sensitive, simple, specific and accurate liquid chromatography tandem mass spectrometry methods were developed and verified for the determination of the

vitamins D metabolites in serum. The sample preparation techniques were kept quick for easy application and the various ionization modes were evaluated that resulted in no major differences while the addition of the methylamine gave increased responses. The use of the derivative PTAD boosted the response and sensitivity of the compounds that were all baseline separated particularly for the dihydroxyvitamin D metabolites. Thus the various vitamin D metabolites can be evaluated together albeit at different concentrations without any major issues due to the design of the methodologies.

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Impact of Seminal Plasma Copper to Retinol Ratio in Infertile Subjects

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Introduction: Infertility is a complex problem that influenced by multifactorial causes. One of known cause is micronutrient and also trace element. Retinol plays an important role in sperm quality through its antioxidant activity to protect spermatozoa against free radical. Copper also suggested have higher potency as a sensitive indicator on sperm quality. Quantification method also play critical role in accuracy and sensitivity. There is no available data that combine these two potential infertility marker as a ratio and its correlation with sperm quality parameters quantified using mass spectrometry. **Objective:** This study objectives were for investigating impact of retinol, copper, and copper to retinol ratio in infertility from seminal plasma quantified by mass spectrometry. **Method:** Seminal plasma was obtained by consecutive sampling of sperm analysis in Prodia Clinical Laboratory consist of 33 normal zoopermia subjects and 20 abnormal zoospermia subjects with mean age 33 years old. Retinol quantified by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and copper quantified by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Data analysis was using SPSS 24. **Result:** There was no significant age difference between normal and abnormal zoospermia subjects (33 ± 4 vs 35 ± 9, p = 0.713). There was significant negative correlation between retinol with number and concentration of spermatozoa (r = -0.472, p = 0.001 and r = -0.522, p = 0.000) and also significant positive correlation between copper with number and concentration of spermatozoa (r = 0.454, p = 0.009 and r = 0.302, p = 0.028). Copper to retinol ratio shown significant positive correlation with number and concentration of spermatozoa (R = 0.517, p = 0.000 and R = 0.470, p = 0.000) which was also significantly higher ratio in normal zoopermia subject compared to abnormal zoospermia (7.6 ± 3.2 vs 2.8 ± 2.7, p = 0.000). Cut off value of ratio suggested that subject have abnormal zoospermia was below 5.2 (AUC = 0.880 95%CI: 0.78 - 0.98). Copper to retinol ratio shown higher AUC compared to copper and retinol alone in infertility (0.880 vs 0.808 vs 0.850). **Conclusion:** Result suggest that retinol and copper have individual potential marker in infertility status based on abnormal zoospermia. Combination of these markers as a ratio give potential impact to distinguish infertility status based on micronutrient and trace element. **Keywords:** Copper, Retinol, Seminal Plasma, Infertility

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Investigation of calibration matrix materials to achieve optimal sensitivity of total testosterone by LC-MS/MS

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Background:
Superior specificity and sensitivity are two benchmarks, which support the analysis of testosterone in serum by LC-MS/MS as the gold standard methodology, especially for females, children and hypogonadal males. According to CLSI C-62A guidelines, "background peaks should be absent or <20% of the peak area for the analyte at the LLMI." In addition, a minimal difference between the internal standard responses of the calibration material and patient samples is essential for accurate concentration calculation. The ideal calibrator matrix would provide the lowest background (Blank) response combined with the highest correlation of internal standard (ISTD) response between patients and blank matrix. The objective is to identify the matrix material most suitable for use as blanks and calibration standards in an assay for the quantitative analysis of serum total testosterone by LC-MS/MS.
Methods:
Eight different types of matrix materials were investigated: charcoal stripped human serum (MSG-4000, Golden West Biologicals), synthetic human serum (SigMatrix, Sigma Aldrich and SMx Serum, UTAK Laboratories), commercially available calibration standard kits which contained a lyophilized serum blank (ChromSystems and Cerrilliant), 5% human serum albumin (Sigma Aldrich), normal saline and 50% methanol. The LLMI target was 2 ng/dL for testosterone. A simple LLE extraction was performed on 200 µl of the calibrator matrix and patient serum, using ethyl acetate:hexane (90:10), followed by flash freezing the aqueous layer and then pouring off and drying the organic layer. The dried extract was reconstituted in mobile phase and 25 µl was injected. Double blank, blank and a calibration standard near the anticipated LLMI were analyzed for each matrix type. An Agilent 1260 series HPLC and 6460 QQQ were used to separate and detect the presence or absence of testosterone in the calibrator matrix and serum samples. A 50 mm x 2.1 µm x 2.7 µm Poroshell 120 EC-18 column was used to separate testosterone and similar structured moieties. Generally, a 3.0 minute HPLC gradient was run from 53% to 56% using a mobile phase of 5 mM ammonium formate in methanol and 5mM ammonium formate in water. Detection in the mass spectrometer was accomplished using positive mode ESI and monitoring two ion transitions, 289.2>97.1 and 289.2 > 109.1 m/z, for testosterone and one for the ^{13}C internal standard, 292.1>112.1 m/z.
Results:
Saline, 50% methanol, SigMatrix and SMx Serum produced the least percentage background peak and most consistency ranging from 2%, to 5%.. The commercial calibrator set, 5% human serum albumin and stripped serum had the largest percent background peaks at 11% to 16%.. Unexpectedly, the human serum albumin had a greater than expected lot variation of 3% and 14% between the two lots. Internal standard response difference between calibrators and patient samples ranged from 12% to 24%, with SMx Serum at 12%.
Conclusions:
The SMx Serum matrix from UTAK Laboratories enabled the optimal assay LLMI by providing the lowest background peak, an acceptable correlation between the testosterone internal standard responses of the calibration standards compared to the patient specimens, the greatest consistency between batches and was most cost effective.

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Evaluation of a High Sensitivity Estrone and Estradiol Assay by LC-MS/MS

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Background: High sensitivity measurement of estrone/estradiol is important as part of the diagnostic workup of delayed puberty in females, disorders of sex steroid metabolism, antiestrogen therapies of breast cancer, and bone health of postmenopausal women. LC-MS/MS based assays are superior to conventional immunoassays with improved sensitivity and specificity. We aim to develop a high sensitivity assay for estrone/estradiol by LC-MS/MS for clinical diagnostic use. We evaluated performance of the assay focusing on sensitivity using several approaches recommended by guidelines. **Methods:** We evaluated options of sample volume and preparation, column chromatography and instrumentation, and constructed a LC-MS/MS method without derivatization. Sample preparation is by SLE+ cartridges using 200 µL serum of adult or pediatric patients. Column chromatography is by 2D reverse phase separation using mobile phases containing ammonium fluoride. A QTRAP LC-MS/MS instrument and ESI in negative mode were used to detect and quantify estrone and estradiol. The assay performance including linearity, sensitivity, specificity, and accuracy was validated for clinical diagnostic use. Sensitivity was further evaluated using CLSI, FDA, ISO, CAP, and EU guidelines. **Results:** The assay sensitivity for estrone/estradiol was in range of 2-10 pg/mL when evaluated by the precision profiles, signal-to-noise ratios, and maintenance of ion transition ratios. The assay was linear up to 1,000 pg/mL for estrone and 2,000 pg/mL for estradiol. Correlation data were y=0.94x-6.8 [LC-MS/MS, Ref Lab 1], n=20, r^2=0.98 for estrone and y=1.0x+1.1 [LC-MS/MS, Ref Lab 2] n=75, r^2=0.98 for estradiol. **Conclusion:** The LC-MS/MS method is highly sensitive and with easy to follow steps in sample preparation. It has satisfactory clinical performance for estrone and estradiol measurement in both adult and pediatric patients and it may have broad clinical application due to its extensive measuring range.

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BIUXX

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Background: The abundance of mass spectrometry data can be beneficial yet overwhelming. Automated platforms many times only provide a numeric value but mass spectrometry platforms provide countless pieces of data as to why that numeric value is analytically valid. Many laboratories recognize the power of these data elements and manually review them to enhance the validity of the fi-

nal value. But this review takes an enormous amount of technologist time. Solutions surrounding auto-data review for mass spectrometry based assays exist but use "static" rules that define acceptable peak characteristics. Unfortunately, static rules may eventually fail entire batches, not because the quality of data has suffered but because the entire assay has shifted in an acceptable way. Many laboratories realized that mass spectrometry assays are dynamic processes which ebb and flow as the method/mass spec/LC/columns age. Because calibrators are generally run with each patient batch, what if the calibrators themselves could calibrate more than just the final sample value? What if these calibrators could also provide "dynamic" information regarding retention time? Relative retention time? IS peak area? If these data elements could be analyzed, then the dynamic rules developed can move with the changes in the assay. This greatly reduces the amount of IT/mass spec department maintenance of specific mass spec assays over time. **Methods:** The data described in the presentation was obtained using a Sciex 5500 Mass Spectrometer coupled with a Shimadzu LC20 Liquid Chromatograph at Providence Regional Laboratories in Portland Oregon. Multiquant was used as the quantitation software. The driver was designed by Data Innovations in S. Burlington, Vermont. This is a beta driver designed to average data elements from any samples designated as "calibrators". These averages are then populated under each patient sample which can then be compared to the individual patient data. Rules are generated from this comparison using specific criteria defined and designed by Providence Regional Laboratories. The rules and scenarios were tested using test patients with artificially integrated mass spectrometry data to mimic potential problems with samples. **Results:** After validation of our in-house opiates LCMS assay using the new driver, we observed a significant decrease in review time. Analytes which required manual review were reduced to less than 15% of the total number of analytes reviewed. Error rates for auto-data review were significantly lower than with manual review of data by technologists. The overall time to completely review each patient batch was reduced by over 50%. **Conclusion:** Dynamic auto-data review is a concept that is not entirely new, but requires individual laboratories to produce highly customized, in-house solutions. There needs to be an easier way for laboratories to access these solutions, preferably using technologies and strategies many labs have already acquired. This new data innovations driver provides a "jump start" to middleware rule writing that can be semi-customized based on the individual laboratory's needs. By elevating and incorporating clinical mass spectrometry data into middleware, we bridge the gap between mass spectrometry and routine automated instrumentation platforms.

B-226

New Card for Blood Collection Anytime and Anywhere

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Background: Non-traditional blood collection is growing rapidly for drug compliance, nutritional and wellness testing. The leading method for blood collection outside a clinical facility is the dried blood spot (DBS) card, but DBS results can be difficult to correlate to plasma levels which are needed for clinical significance. Plasma cards are a new alternative to DBS cards that produce dried plasma spots from a drop or two of whole blood. A new plasma card will be evaluated for use in drug and wellness testing. **Methods:** Human blood was collected in accordance with our IRB protocol. Vitamin D reference standards SRM 968 L1/L2 and SRM 968d L1 were obtained from NIST. Isotope labeled Vitamin D and indomethacin were obtained from Medical Isotopes (Pelham, NH). Vitamin D was analyzed as described in Anal. Chem., **2013**, *85* (23), pp 11501-11508. The Vitamin D reagents were obtained from Novilytic. Vitamin D samples were analyzed on a Sciex 4000 mass spectrometer. Indomethacin was analyzed by Alturas Analytics according to their method presented at the AAPS 2016 Conference in Denver, CO. Samples were analyzed on a Sciex 5500 mass spectrometer. One and two disc plasma cards were obtained from Novilytic. **Results:** Combining two plasma collection discs resulted in a total plasma volume of 10 µL. The lower limit of detection (LOD) for vitamin D2 was just under 2 ng/mL using two plasma collection discs. The LOD for vitamin D3 was also under 2 ng/mL. The CV at the limit of detection for both vitamin D2 and D3 was less than 12%. A single plasma collection disc agreed with the vitamin D3 reference standard at 12 ng/mL to within 7% and two plasma discs combined agreed within 5%. Indomethacin was detectable down to 9 ng/mL with a simple methanol extraction using two plasma collection discs. The bias and the CV for two plasma discs were less than 14% at the limit of the detection, 9 ng/mL. The quality control at 1,200 ng/mL showed excellent results with the bias and CV less than 7% for either a single plasma collection disc or two combined discs. **Conclusion:** A new plasma card enables remote blood collection and fast plasma preparation within minutes. The resulting plasma samples are sufficient to measure vitamin and drug levels at the low ng/mL levels using LCMS.

B-227

Development and validation of a quantitative method for plasma markers of transmethylation and transsulfuration

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Background: Metabolism of methionine (Met), homocysteine (Hcy) and cysteine (Cys) is critical for folate-dependent transmethylation and transsulfuration. Abnormal metabolism of these sulfur-rich amino acids may be associated with genetic or epigenetic factors. The primary markers of defects in remethylation and transsulfuration include not only Met, Hcy, and Cys, but also cystathionine (CSH), S-adenosylhomocysteine (SAH), and S-adenosylmethionine (SAM). Our goal was to develop and validate a comprehensive method for the measurement of these markers in plasma using liquid chromatography with tandem mass spectrometry (LC-MS/MS). **Methods:** Plasma samples prepared in 1M acetic acid were mixed with stable isotope internal standards for each of the measured components. For the sulfur-rich amino acids, a strong reductant, dithiothreitol, was added to yield free forms of homocysteine and cysteine from their disulfides, with subsequent derivatization with ethylchloroformate to prevent oxidation and facilitate efficient liquid-liquid extraction with ethyl acetate. For SAH and SAM sample preparation, phenylboronic acid solid phase extraction and nonafluoropentanoic acid, an ion paring reagent were employed to facilitate separation and ionization. Certified standards were acquired to derive a 5-point calibration curve for each analyte. Calibrators and plasma extracts were analyzed on an Agilent 6460 LC-MS/MS with chromatographic separation achieved on a C18 analytical column, permitting identification and quantitation of the compounds of interest. Precision, accuracy, linearity, recovery, and stability were evaluated. **Results:** The intra-assay and total imprecision coefficients of variation (CV_W, CV_T) (n=24) in plasma samples was determined for Met: 0.8%, 8.7% at 1.7 µmol/dL, and 1.2%, 7.5% at 3.4 µmol/dL; Hcy: 1.2%, 8.6% at 5.0 µmol/dL, and 1.2%, 4.8% at 10.2 µmol/dL; Cys: 1.0%, 10.8% at 16.2 µmol/dL, and 0.4%, 8.9% and at 36.0 µmol/dL; CSH: 5.6%, 19.2% at 0.08 µmol/dL, and 2.7%, 5.5% at 0.30 µmol/dL; SAH:1.0%, 1.8% at 61.0 nmol/L, and 0.9%, 1.2% at 113.2 nmol/L; and SAM: 2.4%, 6.6% at 40.4 nmol/L, and 2.9%, 3.3% at 141.7 nmol/L. Linearity range (n=11) and percent recovery were confirmed in spiked plasma samples for Met: 0.4-4.0 µmol/dL, 95.9%-105.6%; Hcy: 2.0-20.0 µmol/dL, 96.3%-101.5%; Cys: 4.0-40.0 µmol/dL, 96.9%-102.9%; CSH: 0.04-0.40 µmol/dL, 94.0%-104.5%; SAH: 5.0-120 nmol/L, 98.1%-102.9%; SAM: 11.0-300.0 nmol/L, 96.7%-102.3%. Least-squares regression analysis comparing split plasma extracts on the Agilent 6460 LC-MS/MS to a previously validated LC-MS/MS method (n=76) yielded correlation coefficients for Met: 0.892, y = 1.107x + 0.363, range 0.6-3.9 µmol/dL; Hcy: 0.986, y = 1.027x + 0.107, range 3.6-16.9 µmol/dL; Cys: 0.972, y = 1.049x + 0.431, range 8.0-35.3 µmol/dL; CSH: 0.994, y = 1.086x - 0.004, range 0.04-0.31 µmol/dL; SAH: 0.995, y = 1.071x - 2.772, range 10.5-58.7 nmol/L; and SAM: 0.925, y = 0.925x - 4.684, range 48.6-219.8 nmol/L. Adequate stability of all analytes in plasma acidified with 0.1% acetic acid (v/v%) was demonstrated for 10-days stored at 2-8ºC, and 30-days stored at -20ºC. **Conclusion:** This LC-MS/MS method was validated to provide sensitive, precise and accurate evaluation of plasma Met, Hcy, Cys, CSH, SAH, and SAM to guide clinical intervention to improve or normalize methionine metabolism and ameliorate or prevent the potential adverse consequences associated with inadequate methylation and transsulfuration capacity.

B-228

Development and Validation of a LC/MSMS Method for Simultaneous Quantification of Five Vitamin D Metabolites

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Background: There has been a growing interest in Vitamin D and its clinical associations in the last decade. Since it generally correlates well with vitamin D stores of the body and numerous automated immunoassays are readily available, plasma concentrations of 25(OH)D is the most measured metabolite to investigate vitamin D metabolism. However, simultaneous measurement of various forms and isomers of vitamin D metabolites can reveal how vitamin D metabolism is affected in different clinical conditions. Therefore, we aimed to design a method that can analyze the frequently investigated vitamin D metabolites, namely 25(OH)D3, 1,25(OH)2D3, 24R,25(OH)2D3, 25(OH)D2, and 3-epi-25(OH)D3 simultaneously. **Methods:** We developed and validated a high-performance liquid chromatography tandem mass spectrometry (LC/MSMS)-based method by using standards and internal standard solutions for vitamin D metabolites to quantitate each of these in plasma samples. 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and methylamine were

used for derivatization after liquid/liquid extraction, and vitamin D metabolites' derivatives were chromatographically separated using pentafluorophenyl (PFP) and C18 columns. We evaluated performance parameters e.g., matrix effect, carryover, measuring range, limit of quantitation, linearity, imprecision, interferences and accuracy of the method according to the Clinical & Laboratory Standards Institute: Liquid Chromatography-Mass Spectrometry Methods, Approved Guideline (C62-A). **Results:** The method met the linearity and imprecision criteria within the measuring ranges [3.1-100.0 ng/mL for 25(OH)D_3, 15.6-500.0 pg/mL for 1,25$(OH)_2D_3$, 0.6-20.0 ng/mL for 24R,25$(OH)_2D_3$, 1.3-40.0 ng/mL for 25(OH)D_2 and 0,6-20,0 ng/mL for 3-epi-25$(OH)D_3$], chosen in accordance with the clinical decision-making levels of vitamin D metabolites. The validation process was completed with imprecision and accuracy evaluation; coefficient of variations of all metabolites at various concentrations did not exceed 9.3% at all CV classifications (within-run, between-run, within-day, between-day and total) and the highest bias% obtained from the measurement of 4 levels of the certified reference materials (SRM972a) provided by NIST for 25$(OH)D_3$, 24R,25$(OH)_2D_3$, 25(OH)D_2 and 3-epi-25$(OH)D_3$ were (+1,3%), (-3,8%), (-8%), and (-8%), respectively. Reduction of isomeric and/or isobaric interferences on 1,25(OH)2D3 measurement and separation of 25(OH)D3 and 3-epi-25(OH)D3 peaks were established by using two analytical columns together. **Conclusion:** LC/MSMS-based methods have high sensitivity and specificity and can be used to monitor changes on the concentration of vitamin D metabolites simultaneously in various clinical conditions. In the future, they will reach much better performances with improved extraction, derivatization, chromatographic separation and more advanced instruments. Although they are rapidly gaining ground in clinical chemistry laboratories, precise and accurate results can only be obtained with carefully and accurately validated methods due to the complexity of the LC/MSMS systems.

B-229

High Sensitivity Determination of Underivatized Estradiol and Testosterone in Human Serum by LC-MS/MS

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Background: Accurate measurement of estradiol (E_2) at low concentrations is required in postmenopausal women, men, pediatric patients, and to assess the efficacy of anti-estrogen therapies. The same is true for testosterone (T) in women, children, hypo-gonadal men and the need to control anti-androgen therapies. This study aims to develop and validate high-sensitive methods for analysis of underivatized E_2 and T in low volume of human serum. **Methods**: E_2 and d_3-E_2 (internal standard) were extracted from 200 µL of human serum with 1-chlorobutane, and T and d_3-T (internal standard) were extracted from 250 µL of human serum with ethylacetate. Chromatographic separation was performed on a core shell C18 analytical column under specific gradient elution for each analyte, with mobile phases consisting of methanol (phase A) and 0.2 mM ammonium fluoride (phase B). Negative electrospray ionization was used for E_2 to follow the predominant transitions: collision energy (CE) -64, m/z 271→143 (qualifier); CE-50, m/z 271→145 (quantifier), CE -50, m/z 274→145 for d_3-E_2; positive electrospray ionization was used for T to follow the predominant transitions: collision energy +25, m/z 289→97 (qualifier), m/z 289→109 (quantifier) и m/z 292→109 for d_3-T. Raw data of mass chromatograms were collected and processed by specialized software, and weighted (1/X) linear regressions were performed to determine the concentration of the analytes. Validation strategy was adhered to current industrial and clinical guidance. **Results**: Selectivity was assessed with 20 individual native matrices of human serum applying the technique of standard additions: 5 from children under the age of 6 years, 5 from postmenopausal women, 5 from men, and 5 from premenopausal women, at each point, in the range 2 - 1000 ng/L for E_2, and 0.01 - 20 µg/L for T. Normalized matrix effect averaged 89-112% (percent matrix bias: -11 ÷ 12%), imprecision being within 15%. Inaccuracy ranged from -12.0 to 8.9 % within runs and from -14.9 to 12.2 % between runs. Imprecision was up to 12.7% within-runs, and up to 14.8% between-runs. Linearity was assured in the range 1.0 ÷ 1000 ng/L, R^2>0.996 for E_2, and 0.005 ÷ 20 µg/L, R^2>0.995 for T. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 24 h at 4°C, short-term stability at room temperature was proven for 6 h at daylight and 4 h in the dark; stock solution stability and long term stability in serum were documented for 96 days at -20°C. With run time of 6 min, a throughput of over 100 samples per working day could be achieved for each analyte. **Conclusion**: The two methods were validated according to current industrial and clinical requirements and allow the accurate and precise determination of E_2 and T in human serum at very low concentrations.

B-230

Analysis of Serum Androgens and Corticosteroids for Clinical Research by LC-MS/MS

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Background: Here we evaluate an offline automated method for the measurement of serum androgens; testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEAS), and serum corticosteroids: 17-hydroxyprogesterone (17-OHP), cortisol, 11-deoxycortisol and 21-deoxycortisol, enabling steroid profiling for the investigation of metabolic dysfunction biomarkers for clinical research. An LC-MS/MS method was developed using a novel Solid Phase Extraction (SPE) sorbent in 96-well plate format, reducing sample preparation time and removing more matrix interference in comparison to other sample preparation techniques. **Methods:** Certified testosterone, androstenedione, DHEAS, cortisol, 11-deoxycortisol and 21-deoxycortisol reference material purchased from Cerilliant (Round Rock, TX) were used to create calibrators and QC materials in stripped serum purchased from Golden West Biologicals (Temecula, CA). Serum samples purchased from UK NEQAS (Birmingham, UK) for testosterone, androstenedione, DHEAS, 17-OHP and cortisol were analyzed and concentrations were compared to the EQA MS mean for each steroid hormone. 100 µL serum samples were pre-treated with internal standard, methanol and water. SPE was carried out with a Waters® Oasis® PRiME HLB µElution 96-well plate, providing phospholipid removal and allowing direct injection of the SPE eluate. Offline automated extraction was performed using a Tecan® Freedom Evo 100. Using an ACQUITY UPLC® I-Class system, samples were injected onto a 2.1 x 50 mm Waters ACQUITY UPLC HSS T3 column with a pre-column T3 VanGuard™ using a water/methanol/ammonium acetate/formic acid gradient and quantified with a Waters Xevo® TQ-S micro mass spectrometer. **Results:** The developed method was shown to be linear for the serum androgens and corticosteroids. No significant carryover was observed from high concentrations serum samples into serum blanks. A 1:5 dilution was successfully performed on over-range samples for the serum steroids with recoveries ranging from 97-107% with CVs < 7%. Total precision and repeatability on five separate days for low, mid and high QC samples were all ≤ 7.6% CV (n = 30) for all analytes. Analytical sensitivity investigations performed over three occasions demonstrate a CV < 20% at 0.03 ng/mL for testosterone, 0.025 ng/mL for androstenedione, 0.063 ng/mL for 17-OHP, 4 ng/mL for DHEAS, 0.25 ng/mL for cortisol, 11-deoxycortisol and 21-deoxycortisol. S/N (PtP) calculations at each of these concentrations were > 10:1. Matrix Factor experiments demonstrate the internal standard compensates for ion suppression observed in the method, with matrix factor range of 93 - 100% and CVs < 6.7% for the steroid hormones. The method has shown to be analytically selective through separation of isobaric steroid species and matrix specific interferences such as albumin, triglycerides and bilirubin that could affect accuracy and imprecision. Excellent agreement between this analytical method and the EQA LC-MS mean values have been demonstrated with mean method bias of -0.1%, -5.1%, 5.2%, -5.8% and -1.0% for testosterone, androstenedione, 17-OHP, DHEAS and cortisol, respectively. **Conclusions:** We have successfully quantified serum androgens and corticosteroids using SPE with LC-MS/MS for clinical research purposes. This offline automated method demonstrates excellent linearity, analytical sensitivity, selectivity, precision and accuracy, while providing high sample throughput capabilities. For Research Use Only, Not for use in diagnostic procedures.

B-231

Development of an LC-MS/MS method for measurement of human glycated serum albumin.

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Background: Monitoring glycemic control in patients with Diabetes Mellitus is important to avoid many long term complications associated with the disease. In most patients, glycated hemoglobin (HbA1c) testing is used for long-term monitoring. However, in some situations, e.g. those that affect erythrocyte lifespan, HbA1c results may not be accurate. Glycated Albumin (GA) is a good alternative measurement of glycemic control in these situations. The main goal of this study was to develop a mass spectrometry assay for GA quantitation. **Methods:** A QTRAP 6500+ (Sciex) coupled with Shimadzu HPLC system were used. The assay was initially developed using in-vitro glycated human serum albumin samples. The samples were reduced and alkylated with DTT/IAA, then digested with Glu-C enzyme. The information dependent acquisition was used to identify

all glycated albumin sites. The MRM transitions of two peptides RQIKKQTALVE (521-531) and FKPLVEEPQNLIKQNCE (377-393) and their glycated forms (Lys-525 and Lys-378 accordingly) were chosen for quantitation. The developed protocol was further used for serum samples from subjects with and without diabetes. **Results:** There was a linear correlation between our LC-MS/MS and the commercial Asahi Kasei (Tokyo, Japan) Lucica method for human serum samples, r^2 values were 0.91 and 0.86 for Lys-525 and Lys-378 sites respectively. The CV for human serum samples was 5.2%. **Conclusion:** In summary, we have developed and validated a novel method for glycated albumin quantitation in human serum samples.

B-232

Evaluation of the first FDA-cleared LC-MS/MS assay for quantification of 25-hydroxyvitamin D2/D3 and C3-epimers on the Sciex Topaz system.

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INTRODUCTION: Measurement of 25-hydroxyvitamin D (25(OH)D), i.e., the sum of 25(OH)D2 and 25(OH)D3, has long been recognized as most sensitive and specific when measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) versus other methods such as immunoassay. However, LC-MS/MS for 25(OH)D testing is not widely implemented in routine clinical laboratories due to the need for specialized personnel to operate what are generally considered intricate, open-source platforms. We present one of the first published evaluations of the FDA-cleared Vitamin D 200M assay on the Topaz™ LC-MS/MS system with regard to ease-of-use and prevalence of measurable 25(OH)D2 and C3-epimers in a randomly selected patient population at Cedars-Sinai Medical Center. **METHODS:** Two staff members received one-day of training on the Topaz system and Vitamin D 200M assay. 100µL aliquots of 27 adult serum remnants, 6 calibrators, and 3 quality controls were analyzed with the Vitamin D 200M assay protocol and kit. LC-MS/MS results on these samples were compared to Abbott Architect immunoassay results, which measures total 25(OH)D. Accuracy was assessed by evaluating linearity and reportable range with replicate calibrator extractions. Intraday precision was evaluated with 5 replicate injections of 3 extractions of each of 3 QC levels. The Sciex locked version of Vitamin D 200M for 25(OH)D3 and D2 was also used to evaluate patient C3-epimer concentrations. **RESULTS:** Expected linearity and reportable range were demonstrated for 25-OH Vitamin D3 (R=0.996, 4.37-135.0 ng/mL) and 25(OH)D2 (R=0.999, 2.5 -123.2 ng/mL. %CVs for intraday precision (n=15) of controls were as follows: For 25(OH)D3 Q1=2.1%, Q2=2.7%, and Q3=2.7%, which all fell within expected %CVs; and for 25(OH)D2 Q1=4.0%, Q2=4.1, and Q3=3.2%, which were again within expected limits. The average %bias of the Sciex LC-MS/MS method vs. immunoassay was -7.8%. Amongst 27 patient samples, 10 (32%) had quantifiable 25(OH)D2, and 4% had D2 concentrations >20ng/mL. All samples had measurable concentrations of C3-epimer-25(OH)D3 (range: 2.3-7.7 ng/mL); only one had a quantifiable C3-epimer-25(OH)D2 concentration of 2.8 ng/mL, related to their high 25(OH)D2 concentration (45 ng/mL). However, it has been previously demonstrated that the C3-epimer is primarily related to the 25(OH)D3 level; in our patient population we would estimate that this ~8.6% of the 25(OH)D3 concentration as the linear regression equation of the correlation to immunoassay is: y=0.0855x+0.9812. Additionally, 4 samples had C3-epimer-25(OH)D3 >5 ng/mL (i.e., 15% of the study population). **CONCLUSION:** Although other parameters can be used to assess ease-of-use, after one day of training on the Topaz™ LC-MS/MS system, staff could quantify 25(OH)D2/D3 with linearity, reportable range, and precision as expected based on cleared specifications. Correlation to immunoassay was biased by -7.8%, which is to be expected when both 25(OH)D2 and C3-epimers are present at appreciable concentrations. Lastly, the observation of measurable 25(OH)D2 and the C3-epimers in 32% and 100% of patient samples tested, respectively, emphasizes two points: 1) The importance of the specificity offered by the Topaz™ LC-MS/MS system and vitamin D assay in routine hospital populations. And 2) Recognition that C3-epimer is not inconsequential in the adult population and should likely be quantitated, yet many lab-developed 25(OH)D LC-MS/MS assays do not.

B-233

Determination of cadmium, lead, mercury, and nickel in blood for assessment of environmental exposure by inductively coupled plasma mass spectrometry: a comparison with atomic absorption spectrometry and stability test of samples under refrigerated condition

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Background: The purpose of this study is to establish Cadmium, Lead, Mercury, and Nickel analytical methods by Inductively coupled plasma-mass spectrometry (ICP-MS) for investigating environmental exposure to the trace metals in Korean general population and to ensure the continuity with previous national biomonitoring data by atomic absorption spectrometry (AAS) through comparing ICP-MS with AAS. In order to confirm the change with the time delay from sampling to the measurement, the stability of samples under the refrigerated condition was also evaluated. **Methods:** We established and validated the dilution methods for Cd, Pb, Hg, and Ni in whole blood sample using ICP-MS (7900X ICP-MS, Agilent Technologies, Japan). We compared our routine external calibration method and the matrix-matched calibration method with G-EQUAS (German External Quality Assessment Scheme) materials with assigned value. Whole Blood samples from 100 healthy Korean population were collected in trace element EDTA tubes (BD, USA). Cd, Pb, and Hg levels were determined using both ICP-MS and AAS (Cd; AAnalyst 800, Perkin Elmer, Singapore, Pb; 240Z AA, Agilent Technologies, Australia, and Hg; DMA 80, Milestone, Italy). The stability test under the refrigerated condition was carried out for Cd, Pb, Hg, and Ni with 10 patient samples on the 1st, 3rd, 7th and 21st days after blood sampling. **Results:** Limit of quantifications were 0.31 µg/, 2.11 µg/dL, 1.06 µg/L, and 0.99 µg/L for Cd, Pb, Hg, and Ni, respectively. The average within-batch and total coefficients of variation were below 10 % in all analytes. The percent bias against the assigned value of two levels of G-EQUAS material were 2.51, 4.90 for Cd, -1.00, 0.04 for Pb, and -1.28, -3.36 for Hg with external calibration and 5.26, 4.70 for Cd, -2.24, -0.07 for Pb, and 0.22, -0.80 for Hg with standard addition method. The differences between ICP-MS and AAS method for Cd, Pb, and Hg were statistically significant ($P < 0.001$). The regression equation for the comparison between ICP-MS (y) and the AAS method (x) was $y = 1.061x + 0.018$ ($r^2 = 0.964$) for Cd, $y = 0.910x - 0.242$ ($r^2 = 0.855$) for Pb, and $y = 0.978x - 0.048$ ($r^2 = 0.985$) for Hg. The average of differences of ICP-MS against AAS were +11.6 % for Cd, -21.8 % for Pb, and -3.4 % for Hg. In regression analysis for the stability test, the probability of F of all items was> 0.05. **Conclusion:** Our ICP-MS method for the determination of Cd, Ob, Hg, and Ni showed a good performance, and the ICP-MS results could be converted to be equivalent to the previous AAS results, so it can be used for biomonitoring in general population. There was no significant change in the stability according to the shelf life, therefore, there is no concern about the quality of specimens during refrigerated storage and transportation.

B-234

Development of an LC-MS/MS method for quantifying DNA methylation in whole blood for assessment of hematologic malignancies.

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Background: DNA methylation is a dynamic physiologic mechanism for silencing genes. In cancer, these epigenetic modifications can be dysregulated to be advantageous for tumorigenesis. Numerous cancers, including some hematologic malignancies, are well-described in the literature as having hypermethylator phenotypes. Clinically, DNA methylation status has been of recent interest in acute myeloid leukemia (AML) as a new paradigm of prognostic stratification, often showing increased survival. Despite this, a clinically validated method for assessing total genomic methylation of these cancers in clinical samples has yet to arise. Our research provides a mass spectrometry-based method for quantifying methylated DNA extracted from whole blood samples for assessment of hypermethylator phenotypes in hematologic malignancies. **Materials and Methods:** Analysis of remnant clinical samples was approved by the UCSF Institutional Review Board. Genomic DNA was extracted from 200uL of whole blood, concentrated, and enzymatically hydrolyzed into nucleosides. Chromatographic separation of analytes was performed with a Kinetix C18 column (50x3mm, 2.6um; Phenomenex). Data was acquired on a QTRAP 4500 (SCIEX) using positive-ion mode multiple reaction monitoring. Quantifier and qualifier fragment ion transitions for 2'-deoxycytidine (C), 5-methyl-2'-deoxycytidine (5mC), and deuterated internal standards (Toronto Research Chemicals) were used to measure peak area. A calibration curve was constructed for concentrations 10 to 2000 ng/mL. Appropriate DNA hydrolysis

was confirmed each run with native and methylated DNA oligomer controls (ZYMO Research). Percentage of DNA methylation was calculated from the concentrations of 5mC divided by the sum of 5mC and C measured in each sample. Validation of this method was performed with 5 separate extractions from analyte-spiked whole blood for a 10-point calibration curve with 3 quality controls spanning the range of the curve. **Results:** Inter-assay calibration curves (n=5) were linear and reproducible over 10-2000 ng/mL (r^2>0.99). Accuracy, as measured by percent error from nominal value between inter-assay calibrator and QC concentration mean values ranged from 0.74-11.55%, with an average percent error of 5.95%. Inter-assay coefficient of variance (CV) for each calibrator and QC ranged from 4.14-8.10%, with an average CV of 6.08%. Calculation of DNA methylation in healthy control whole blood showed percent cytidine methylation of 3.08%. **Conclusion:** This method is novel in that it provides a clinical diagnostic tool for assessing total genomic methylation from whole blood, which can potentially be used to assess methylation status of hematologic malignancies. Further analytical and clinical studies using this method will be performed to confirm and explore prognostic correlations that have been preliminarily reported for hypermethylator phenotype hematologic malignancies, specifically AML.

B-235

Measurement of Serum Iohexol by LC-MS/MS to Assess Glomerular Filtration Rate in Kidney Transplant

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Background: We have developed a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay to measure iohexol, a filtration marker, for accurate glomerular filtration rate (GFR) determination as part of living kidney donor evaluation. In the recently published guidelines by Kidney Disease Improving Global Outcomes (KDIGO), measured GFR (mGFR) is recommended as confirmatory test for estimated GFR (eGFR) to better assess the long-term end-stage renal disease (ESRD) risk for donor candidates. The current mGFR method implemented in our hospital involves the use of a radioactive marker (^{125}I-iothalamate), which poses potential safety risk and often suffered from supply shortage. The purpose of the current study is to develop a simple yet sensitive method to allow the use of the non-radioactive iohexol as an alternative. **Methods:** After a single bolus of iohexol (5mL Omnipaque 300™), three blood samples were collected at 120, 180 and 240 minutes' interval after injection of iohexol. Upon separation of serum from blood, iohexol concentrations were then measured by LC-MS/MS. From serum iohexol levels and the administration dose, GFR would be calculated using the one-compartment open model system corrected according to the Brochner-Mortensen formula. Standard GFR would also be calculated by correcting GFR to body surface area (BSA). To measure serum iohexol, a quick sample extraction method was developed using strong acid protein precipitation. The LC-MS/MS method was developed on Thermo Vanquish UHPLC system coupled with TSQ Endura triple quadruple mass spectrometer. Iohexol and d5-iohexol, the internal standard, were simultaneously eluted by a Kinetex EVO C18 column (5µm, 50*3.0 mm), using a 2.5-minute gradient of 0.1 % formic acid in water and methanol. Two multiple reaction monitoring (MRM) transitions were set for iohexol: 822.0◊804.0 (for quantitation) and 822.0◊603.0 (for confirmation); and one MRM transition is set for d5-iohexol: 827.0◊809.0. Simultaneous peak integration and quantitation for iohexol were achieved automatically using the pre-installed TraceFinder™ Software. **Results:** Validation study shown great linearity ($R^2 \geq 0.99$) across the analytical measurement range (AMR) from 5 to 1000 µg/mL. Assay within-day and between-day precisions were assessed at three different QC levels, with coefficient of variation (CV) less than 15% achieved for each level. To assess the assay accuracy, results from 20 iohexol-spiked serum with concentrations across the AMR were compared to that obtained from a reference laboratory. The regression analysis shown a slope of 0.995 (0.968 to 1.023, 95% CI), an intercept of 11.092 (-1.447 to 23.632, 95%CI), and the standard error estimate of 14.817. The correlation efficiency is 0.998 with percent bias of 2.4%. Further validation study, including ion suppression and specimen stability, is currently underway. **Conclusion:** In summary, we have developed a new LC-MS/MS assay to accurately measure iohexol, providing a safer GFR assessment method that is easy to implement, from which both our patients and clinicians can benefit.

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Determination of urinary metabolites of gasoline using LC-MS; method validation

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Abstract

Background: Biological monitoring (BM) is an evaluation of occupational exposure to volatile organic compounds in gasoline and an important measure for the prevention and protection of occupational intoxication. Objective: We aimed to develop a method to simultaneously assess 4 metabolites of gasoline in urine samples of exposed workers; trans, trans-muconic acid (MUC), mandelic acid (MAN), hippuric acid (HIP) and orthomethylhippuric acid (MHP) which are metabolites of benzene, ethylbenzene, xylene & toluene (4 main constituents of gasoline) using Liquid Chromatography-Mass Spectrometery (LC-MS). Method: The developed method was suitable for quantitative analysis of the targeted metabolites in the urine of gas stations workers. FDA regulations for analysis in biological fluids were followed for validation. Procedure: Standards of HIP, MHP, MAN and MUC were provided from Sigma-Aldrich, St. Louis, Missouri, USA. Urine samples & standard solutions were used. Shim-pack® XR – ODS II column was used as stationary phase with a gradient eluting solvent of 1% formic acid and acetonitrile. The metabolites were resolved with retention times of 3.1, 5.8, 6.2 and 11.1 min for MUC, MAN, HIP and MHP, respectively. The MS detector was APCI (atmospheric pressure chemical ionization) adjusted to negative SIM (selected ion monitoring) mode to eliminate the interference and enhance sensitivity. The selected m/z values were 141, 151, 178, and 192 for MUC, MAN, HIP and MHP, respectively. Results: Accuracy: recoveries ranged from 92.87-105.01 % at three levels of concentration, and from 82.5 – 88.9 % at the limit of quantitation. Precision: The within-run coefficient of variation (CV) ranged from 0.580% to 8.138% for all analytes. The repeatability CV ranged from 1.052% to 6.316%. Measuring range & linearity: The limits of detection were 0.117, 0.251, 0.139, and 0.109 µg/mL, and the limit of quantification values were 0.355, 0.759, 0.422, and 0.329 µg/mL for MUC, MAN, HIP and MHP, respectively. The method was linear up to 20 µg/mL for all analytes. No extraction procedure was required for analysis in urine and being a simple non-invasive method, it could be applied for routine check ups of workers in gas stations. Matrix ion suppression was avoided using many strategies including sample dilution, APCI ionization. The method was sensitive and selective to simultaneously analyze the four metabolites in presence of possible urine interferences as albuminuria & hemoglobinuria. Stability parameters were also tested at room temperature, refrigeration, 3 freeze-thaw cycles & long term storage at -80°C Conclusion: The developed method was suitable for quantitative analysis of the targeted metabolites in the urine of gas stations workers. It will be further used in a study to assess vestibular system dysfunction among workers exposed to gasoline. Metabolites level will be assessed in urine samples pre and post shift. Urinary metabolites will be normalized analyte concentration-to-urine creatinine concentration to compensate for fluctuations in absolute concentration related to physiologic variation as urine dilution or concentration.

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Candidate reference method of serum thyroxine using Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry

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Background: There are some reference methods to determine serum total thyroxine by isotope dilution gas chromatography mass spectrometry and liquid chromatography mass spectrometry which are time consuming and complicated. A need exists for a simple reference method that can be easily adopted to verify the accuracy of serum thyroxine measurements. So candidate reference methods involving isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) for total thyroxine were established. **Methods:** Serum samples were sampled by weight and the $^{13}C_6$-thyroxine internal standards were added volumetrically using automated dilutors,followed by equilibration,protein precipitation,and cation exchang solid-phase extractions(SPE). After SPE,the eluates were evaporated to dryness under nitrogen and then the evaporated residues were reconstituted to prepare samples for liquid chroma-

tography-mass spectrometry electrospray ionization (LC/MS-ESI) analysis using electrospray for ionization (ESI). For separation, a Zorbax Eclipse XDB C18 column was used with a mobile phase consisting of 0.05% formate in water-methanol (30:70 by volume) for positive ions. The quantitative ion transitions of $[M+H-HCOOH]^+$ at *m/z* 777.7→731.6 and *m/z* 783.7→737.6 were monitored for thyroxine and $[^{13}C_3]$-thyroxine, respectively. The qualitative ion transitions of $[M+H-HCOOH-I]^+$ were at *m/z* 777.7→633.8 and *m/z* 783.7→639.8, respectively. **Results:** The within-run,between-run and total coefficients of variation were:0.60%(0.35%~0.82 %),0.54%(0.27 %~1.23 %) and 0.84%(0.57%~1.37 %),respectively. The analytical recoveries ranged from 99.6% to 100.7%. The limit of detection was 0.12 nmol/L (S/N ratio 3:1) and the limit of quantification was 0.41nmol/L (S/N ratio 10:1) for thyroxine in human serum. The results of analyzing the certified reference material of German Societies for Clinical Chemistry (DGKC) CRM21201 and CRM21202 showed biases of -0.30% (ranged from -0.13% to 0.73%). **Conclusion:** Isotope dilution LC/MS/MS method for serum thyroxine has been developed. The method is less sample volume demand and less time-consuming and may be used as a candidate reference method. This method was been used in international laboratory comparison including RELA (IFCC) and domestic Reference measurement comparison of EQA. Results showed that this ID-LC/MS/MS method was well-characterized for serum thyroxine with a theoretically sound approach, demonstrated good accuracy and precision, and low susceptibility to interferences qualifies as a candidate reference method. Use of this reference method as an accuracy base may reduce the apparent biases in routine methods along with the high interlaboratory imprecision.

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Clinical Validation and Implementation of a High Throughput Method for Measuring Whole Blood Lead Levels Using an Alkaline Digestion and ICP-MS

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Background: Lead is a cumulative toxicant from environmental exposures with effects on virtually all organ systems including the CNS, liver, gastrointestinal, renal, and hematological systems. Of great clinical concern are the deleterious effects of lead exposure on neurocognitive function in pediatric populations. Low blood lead levels (<5ug/dL) have been shown to affect cognitive function and these effects appear to be irreversible. Atomic Absorption Spectroscopy (AAS) is the most prevalent method for lead testing. Though AAS is adequate for detecting levels >5ug/dL, it is suboptimal for detecting low blood lead levels, uses highly corrosive solvents, and is not conducive to a high throughput environment. We validated a method for whole blood lead testing using Induction Coupled Plasma Mass Spectrometry (ICP-MS) that is sensitive, precise, accurate, and allows for high throughput testing in a CLIA environment. **Methods:** Commercially available lead standards were used to spike into donor blood as well as a matrix matched sample medium (7.5g/L sodium chloride) devoid of lead. Results were verified against commercially available whole blood quality control material (UTAK), CAP proficiency testing material, and clinical specimens that were analyzed in our laboratory using a method that has full approval for patient care (AAS). Whole blood (100uL) was diluted 1:40 in a solution containing 4% butanol, 1% tetramethylammonium hydroxide, 0.01% EDTA, and 0.01% Triton X-100. The diluent was designed to reduce matrix concentration, increase pH, solubilize lead, and minimize the memory effects that can be seen in some ICP-MS methods. The sample is automatically introduced into a 1mL sample loop using a high-speed sample introduction system (ISIS-3; Agilent Technologies) that was optimized for throughput and low carryover, atomized, and subsequently quantified by ICP-MS (Agilent 7900) using the sum of the 3 naturally occurring lead isotopes (206, 207, and 208). An internal standard mixture containing bismuth (209), lutetium (175), and terbium (159) are continuously infused at a 1:10 ratio relative to the primary flow. **Results:** A 1:40 dilution was sufficient to reduce the matrix concentration for robust analysis of a large batch of samples (up to 150 per batch) and still meet the desired limits of detection (0.1ug/dL). The method is linear over 4 orders of magnitude between 0.1 to 100ug/dL with low carryover (<0.25%). The intra- and inter-run imprecision estimates are 2.6% and 3.1% for the lower end of the analytical measurement range (1.8ug/dL) and 1.3% and 3.6% for the upper end of the analytical measurement range (59.7ug/dL) respectively. Accuracy is 84% at 0.1ug/dL and >98% at concentrations greater than 0.3ug/dL. The 3 internal standards are all linear between 1ug/dL and 1000ug/dL and all were accurate for normalization of ionization variability. The method correlated well to AAS (R^2=0.98) as well as CAP proficiency results with no fixed or variable bias. **Conclusion:** We successfully validated and implemented a method in a large university hospital for measuring blood lead by ICP-MS with sensitivity down to 0.1ug/dL using alkaline conditions that can be used for high throughput heavy metal testing.

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Determination of bottled mass of Angiotensin I in candidate Standard Reference Material 998a by amino acid analysis

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Background: The National Institute of Standards and Technology (NIST) has offered a Standard Reference Material (SRM) 998 Angiotensin I (AT-I) for use in the calibration and standardization of renin functional assays. Each vial of this material contained 0.5 mg of AT-I and was reconstituted by adding a desired quantity of solvent. A replacement lot has been procured having an expected mass of 5 mg in each bottle. The purity of the bulk material was assessed prior to packaging and was determined to be 789 mg/g with expanded uncertainty of 58 mg/g (k=2) using amino acid analysis. The current study seeks to determine the average mass and variation of AT-1 as vialed. The true value is critical to determining the reconstitution protocol for this material, whether to add a known amount of solvent or to weigh out milligram quantities for solubilization.**Methods:** Six bottles of the material were selected and approximately 1 mL of 0.01 M hydrochloric acid was added and the mass recorded. The samples were diluted approximately 160-fold and two replicates were taken of each diluted sample. The concentration of AT-1 was determined via amino acid analysis (AAA) using double isotope-dilution tandem mass spectrometry after gas phase hydrochloric acid hydrolysis. Samples were spiked with isotopically labeled free amino acids (isoleucine, leucine, phenylalanine, and valine) either before (n=3) or after (n=3) hydrolysis. Calibration was accomplished using unlabeled amino acids also spiked with labeled amino acids, both without acid hydrolysis. The mass in each bottle was calculated using the determined concentration and known solvent addition and then corrected for purity. **Results:** Values were assigned using linear regression of an external ratiometric calibration curve for each individual amino acid. The regression coefficient was found to be > 0.99 in all cases with slope and y-intercept values close to unity and zero, respectively. The average mass determined for AT-1 was 5.46 mg (5.0 %cv) and 6.00 mg (4.1 %cv) for the groups for which the internal standard was added either pre-hydrolysis or post-hydrolysis, respectively. The means of the two groups were not shown to be different (t-test, $p > 0.05$) and the results were combined to yield an average of 5.7 mg (6.6 %cv). **Conclusion:** The evaluation of the mass contained within each bottle of candidate SRM998a, Angiotensin-I was performed using amino acid analysis and the variation was determined. The bottle to bottle variation shown may be acceptable for use as a calibrant prepared by fixed volume addition in functional assays but will require investigation into each specific case. The larger mass contained in SRM998a will offer the additional ability to measure out specific quantities of material to avoid the bottle-to-bottle variability.

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Fully automated high-throughput urinary creatinine method by liquid chromatography tandem mass spectrometry

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Background: Creatinine is a by-product of muscle metabolism that is excreted in urine at a constant rate through glomerular filtration, and its concentration in urine is proportional to muscle mass. Reliable creatinine measurements are important to evaluate kidney function and for normalizing urinary analyte concentrations. There is a need for specific and sensitive measurements of creatinine to ensure results are accurate and reliable. To address this need, we developed a high-throughput, routine mass spectrometry-based method that requires small sample volumes and provides high accuracy. **Methods:** A high-throughput, fast and accurate liquid chromatography tandem mass spectrometry method for the quantitation of human urinary creatinine was developed and validated. Sample preparation was fully automated including decapping of cryovials, sample ID scanning, and sample aliquoting and processing. Sample processing was performed using a liquid handling system and 96-well plates. This allows for processing of over 600 samples in 8 hours. Quantitation was performed using a stable isotope-labeled internal standard. Multiplexed chromatographic separation of creatinine was achieved within an one-minute run on two Waters Aquity UPLC HSS C18 SB 1.8µm, 2.1x50 mm columns and followed by tandem mass spectrometry on a Thermo Electron triple quadrupole Quantum mass spectrometer in positive electrospray ionization mode. The precursor and product ions of creatinine and D3-creatinine were monitored in selected reaction monitoring mode using the following transitions: m/z 114→44 (quantitation ion (QI) for creatinine), m/z 117→47 (QI for D3-creatinine), m/z 114→72 (confirmation ion(CI) for creatinine) and m/z 117→75 (CI for D3-creatinine). **Results:** Method validation results showed great reproducibility with a within-run precision of 3.59%, 3.49% and 2.84% and among-run precision of 4.01%, 3.28% and 3.57% for low, medium and high quality control materials, respectively. The calibration curve

was linear from 7.5 to 300 mg/dL (R^2=0.9999). Matrix effects were studied in four different matrices and found to have minimal impact on the method with a 95.15 % mean matrix effect observed. Analytical specificity was achieved by chromatographically separating creatinine from potentially interfering creatine within a one-minute run and monitoring the QI/CI ratios in samples. The method showed excellent accuracy with a bias of -2%, -0.8% and -1.1% for three levels of NIST certified reference material. **Conclusion:** A simple, accurate and high-throughput method was successfully developed for measuring urinary creatinine in human urine samples.

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Consumption of Movantik (Naloxegol), an opioid antagonist, results in detection of naloxone in confirmatory urine drug testing

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Background: Many patients that receive chronic opioid therapy suffer from constipation, one of the most common side effect of opioid. Movantik (naloxegol) is an opioid antagonist that is recently introduced in the market to treat opioids-induced constipation and contains naloxegol, as the active ingredient. Naloxegol is a pegylated (polyethylene glycol-modified) derivative of α-naloxol. Confirmatory methods of urine drug testing are known to have high specificity and producing minimum false positive results compared to the screening methods. In pain management clinics appearance of naloxone in the confirmatory urine drug testing report of patients that are prescribed movantik may mislead the clinicians. This study was conducted to investigate the presence of naloxone in the urine of patients that consume movantik in pain management clinics. **Methods:** In a retrospective study the presence of naloxone in the urine of 36 patients that had consumed movantik in pain management clinics of Houston, Texas was investigated. The presence of naloxone was tested using a dilute and shoot liquid chromatography mass spectrometry (LC-MS) method. In concurrence the urinary concentration of naloxone was evaluated in four volunteers that took one pill of movantik. The presence of naloxone in movantik pills was assessed using liquid chromatography mass spectrometry. **Results:** Naloxone was detected in the urine of 34 individuals that were prescribed movantik. All patients observed were also prescribed opioids. Urinary concentration of naloxone showed a bimodal distribution with a mean of 28 ± 20 ng/ml for 26 patients and 133± 49 ng/ml for 8 patients. Consumption of one pill of 25 mg movantik resulted in the detection of naloxone in the urine of four volunteers one hour after taking the pill. Naloxone was not detected 24 hours after the pill consumption. The peak of urinary concentration of naloxone in the volunteers` urine was almost 20 ng/ml. Analysis of movantik pill by liquid chromatography mass spectrometry demonstrated very low concentration of naloxone. **Conclusion:**This study demonstrated that consumption of movantik leads to appearance of naloxone in the urine of patients undergoing opioid therapy in pain management clinics.

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Evaluation of Substance Abuse and Mental Health Services Administration (SAMHSA) pH criteria for definition of adulterated and invalid result specimens in confirmatory urine drug testing.

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Background: Federally-regulated toxicology laboratories that mainly perform workplace urine drug testing utilize certain criteria defined by Substance Abuse and Mental Health Services Administration (SAMHSA) to interpret urine validity tests and report the specimen as valid, dilute, adulterated, substituted and invalid result. If urine specimen pH is ≤ 4 or ≥ 11 the specimen is labeled as adulterated and when the pH is between 4-4.5 or 9-11 the specimen is called invalid result. In contrast to screening immunoassay methods, liquid chromatography mass spectrometry are not prone to many interferences. We tested this hypothesis whether SAMHSA pH criteria for definition of adulterated and invalid result are applicable for both screening and confirmatory urine drug testing. **Methods:** Drug free urine specimens were spiked with common drugs that are tested in clinical toxicology laboratories and the specimen pH altered to a range from 1.6 to 14. The urine specimens were tested with both screening (immunoassay) and confirmatory (liquid chromatography mass spectrometry) using Beckman coulter AU680 Siemens reagents and Sciex 4500 dilute and shoot method, respectively. The confirmatory method measured the concentration of 87 drugs, while the screening method assayed the presence of 9 drugs (amphetamines, barbiturates, benzodiazepine, THC, benzoylecgonine, Methadone, opiates, oxycodone and phencyclidine). Urine specimens were directly used for immunoassay methods. However, the specimens for the confirmatory method were first prepared in a process that included hydrolysis using beta-glucuronidases and dilution and then injected to the machine. The concentration of drugs in urine specimens with a pH of 6.8 was used as the base line and other specimens were compared to these specimens. Alterations greater or lesser than 20% was defined as significant changes in drugs concentration. The drugs concentrations were selected at the cutoff concentrations. **Results:** Concentration of drugs measured by screening immunoassay method did not significantly change when specimen's pH adjusted between 4.5 or 9, reference range for urine pH. In addition, no changes in the concentrations of drugs were detected when specimen's pH were adjusted in the SAMHSA defined pH for invalid result (4-4.5 or 9-11). In specimens with pH ≤ 2, concentrations of benzodiazepines, opiates and phencyclidine were significantly reduced using immunoassay method. The specimens that had a pH ≥12 demonstrated alteration in concentration of benzoylecgonine, THC, methadone, and phencyclidine, when measured by immunoassay method. Amphetamine, barbiturate and oxycodone concentrations did not change in any of tested pH in the immunoassay method. No significant changes in the concentrations of the 88 drugs in the tested pH were detected in the confirmatory liquid chromatography mass spectrometry method except for cocaine, 6 monodactyl morphine, flunitrazepam, methylphenidate and buspirone, which showed a reduction in pH ≥12. **Conclusion:** In contrast to screening immunoassay methods of urine drug testing, the urinary concentration of drugs tested with confirmatory liquid chromatography mass spectrometry method are not altered by changes in pH except for cocaine, 6 monodactyl morphine, flunitrazepam, methylphenidate and buspirone.

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Quantitative analysis of organic acids in plasma using acidified methanol extraction and detection by gas chromatography-mass spectrometry

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Background and objectives: Plasma contains a variety of compounds, and represents a challenging matrix for organic acid analysis. We developed and validated a method based on liquid-liquid extraction using acidified methanol and detection by gas chromatography mass spectrometry. This method allows for the analysis of ten organic acids: lactic, pyruvic, succinic, 3-hydroxybutyric, acetoacetic, 2-keto-3-methylvaleric, 2-ketoisocaproic, 2-ketoisovaleric, glutaric, and citric. **Methodology:** Organic acids are extracted by deproteinizing with acidified methanol, oximated to preserve otherwise unstable short chain ketoacids, and converted to volatile trimethylsilyl (TMS) derivatives before analysis by gas chromatography-mass spectrometry (GC-MS). Compound identification is obtained by retention time and characteristic fragmentation spectra using Agilent MassHunter software. Organic acids are quantified using a six-point calibration curve with 2-ketocaproic acid as internal standard. **Results:** Analytes are linear within the following ranges: lactic acid = 400-6000 uM, pyruvic acid = 10-1000 uM, succinic acid = 10-500 uM, 3-hydroxybutyric acid = 50-1500 uM, acetoacetic acid = 50-1000 uM, 2-keto-3-methylvaleric acid = 5-500 uM, 2-ketoisocaproic acid = 5-500 uM, 2-ketoisovaleric acid = 10-500 uM, glutaric acid = 5-300 uM, and citric acid = 30-800 uM. Intra-assay variability (n=6 in 2 experiments) was <15% for most analytes, and inter-assay variability was <30% (n=3). Recovery in matrix was assessed by testing plasma of 10 healthy donors before and after spiking with a mixture of standards. Recovery of spiked analyte was calculated after accounting for donor endogenous analytes. Recovery was 77% or greater for most analytes. Measurement of lactic, pyruvic and 3-hydroxybutyric acids by GC-MS were comparable to alternative, single-analyte methods (n=10, slopes = 0.913-1.062, R^2 = ≥0.9446). **Conclusion:** We have developed a robust analysis of ten organic acids in plasma. Although this test is not recommended as a routine test for inherited disorders of metabolism, it is utilized in addition to other biochemical genetics testing in some patients.

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Investigating the interferences of lidocaine and its primary metabolites for cocaine metabolites using liquid chromatography mass spectrometry

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Background: Cocaine is a commonly abused drug in the United States that can be also prescribed as a local anesthetic to block nerve impulses. Lidocaine is a locally applied anesthetic in the treatment of arrhythmias. There is a growing concern in the scientific community about the impediment caused by lidocaine in detec-

tion of cocaine in patient populations. The common method for detection of drugs and their metabolites in urine samples are urine drug screen (USD) immunoassays. USD immunoassays are quick and inexpensive; however, they are prone to false-positive results that could affect individual's life, employment and legal citations. In this study, we aim to determine if lidocaine exposure during routine medical procedures can interfere with cocaine or its metabolites, particularly benzoylecgonine. **Methods:** We developed a LC-MS assay to measure lidocaine primary metabolite, two metabolites of cocaine and two metabolites of a common cocaine adulterant. We applied this method to analyze urine from 300 volunteer patients prescribed for lidocaine. The LC-MS assay was developed on a Waters TQD UPLC and detector using a CN column. The mobile phase's compositions were 2mM Ammonium acetate and 0.1% formic acid in either water or methanol. Data was analyzed using Waters MassLynx software. **Results:** Initial results of the first 25 samples show no cross-reaction between lidocaine and any of cocaine metabolites. The assay was linear from 10-2000 ng/mL for all the compounds tested. The total assay precision was less than 15% for all the analytes. The LoD, LoQ and LoB concentrations were 2, 5 and 0.5ng/mL, respectively. The assay is in progress for the rest of our patient samples. **Conclusion:** We developed this assay to help to support or refute the claim that lidocaine and its primary metabolites (nor-lidocaine) cause false-positive reactions with cocaine and its metabolites. This assay should be useful in evaluating immunoassay drug screens for false positivity due to lidocaine metabolites.

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Quantitative Analysis of Iohexol and Iothalamate in Urine and Plasma using LCMS for clinical research use

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Background: Iothalamate and Iohexol are triiodinated derivatives of benzoic acid that are used to assess renal function particularly the kidneys glomerular filtration aspect. The compounds are cleared from the body very rapidly with a clearance rate of 8-11 hours. A simple, sensitive and specific LC/MS/MS analytical method was developed for the quantitation of Iothalamate and Iohexol which are light sensitive using protein crash in plasma and a dilute and shot in urine sample preparation techniques. This easy method achieved good analyte recovery, post-extraction cleanliness and is capable of the sensitivities to quantitate the iohexol and iothalamate in urine and plasma over their dynamic range in bot matrices despite some challenging issues. Method: A Thermo Fisher TSQ Quantis tandem mass spectrometer in positive Electrospray mode and a Thermo Fisher Vanquish Horizon HPLC system were utilized. 200 ul of urine and plasma were used for the analysis of these compounds. Various columns were evaluated and a Thermo Fisher Accucore C18 50 x 2.1 mm, 2.6 um with a water:acetonitrile mixture containing 0.1% formic acid achieved baseline chromatographic separation in less than 6 minute run time for all compounds. Quantitative analysis was performed using scheduled reaction monitoring (SRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using quality control materials and serum samples. Results: Good linearity and reproducibility were obtained with the concentration range from 0.01 to 500 ug/ml for the iothalamate and iohexol with a coefficient of determination >0.995 for both sample preparation. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to range initially from 0.01 and 0.05 ug/ml. Excellent reproducibility was observed for both compounds (CV < 10%) and all techniques and configurations. Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of iothalamate and iohexol in urine and plasma using ioversol as an internal standard. The sample preparation techniques are quick and easily applied for high throughput analysis and included protein precipitation in plasma and urine dilution but improvements are being investigated and the method demonstrates that it is appropriate for GFR determinations and can be used to measure GFR in renal transplant populations.

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Development and Implementation of One-Step, Broad-Spectrum, High-Sensitivity Drug Screening by Tandem Mass Spectrometry in a Pediatric Population

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Background: Urine drug screening by immunoassay is common in pediatric clinical settings. Cross-reactivity, limited scope, and high limits of detection lead to high rates of false positive and negative results. False-positive results are discoverable using higher level analytic techniques such as mass spectrometry. False-negative results last forever. Errors in drug screening, particularly in newborns, have far-reaching medico-legal implications. To circumvent these problems, our laboratory has developed and implemented an LC-MS/MS technique designed to replace immunoassays in urine drug screening. Our objective was to describe the impact of this technique at a large, urban, academic, pediatric teaching hospital and compare it retrospectively to results obtained using our previous immunoassay first-confirmatory approach. **Methods:** Immunoassay screens were performed using a Cobas 6000 system and confirmed by mass spectrometry at a reference laboratory. LC-MS/MS profiles were performed using a Waters Acquity UPLC system equipped with TQ Detector. Urine specimens were diluted with acetonitrile containing 5 internal deuterated drug standards prior to injection. Drug identification was based on detection of precursor ion at an appropriate retention time and the yield of two fragment ions in a ratio characteristic of standard drug. We conducted a retrospective analysis of 4258 pediatric drug screens performed a year before and after the implementation of LC-MS/MS. We reviewed the medical record from the patients of all 1139 samples that tested positive during the two-year surveillance period. We extracted presumptive positive immunoassay results and subsequent confirmatory data and tabulated the identities of all compounds identified by screen-confirm algorithm or the one-step LC-MS/MS approach. **Results:** Prior to LC-MS/MS, 1272 drug screens were performed by the immunoassay in the general pediatric population. Twenty-one percent of these urine specimens were presumptively positive. Of these, 86% compounds were confirmed by MS making the false positive rate 14%. The 3 most prevalent drugs confirmed were THC (44.3%), morphine (18.2%) and amphetamine (18.2%). After implementation of the one step LC-MS/MS approach, 2322 drug screens were performed. 676 (29%) were positive and 28 different compounds were detected. The 5 most prevalent were THC (14.4%), amphetamine (11.5%), fentanyl (7.6 %), benzoylecgonine (7.5%), and morphine (7.4%). In the nursery population, 394 drug screens were performed by immunoassay and 144 (37%) were presumptively positive. Of these presumptive results only 40% were confirmed by MS. The false positive rate of 60% was largely due to high rates of false positive THC detection. Morphine (32.9%), methadone (26.3%) and benzoylecgonine (10.5%) were the most commonly confirmed compounds in this patient cohort. After implementation of LC-MS/MS only testing, 270 drug screens were performed and 48 (18%) were positive. The five most prevalent compounds detected were benzoylecgonine (17.0%), morphine (14.8%), methadone (13.6 %), EDDP (7.5%), and oxycodone (9.1%). **Conclusion:** Primary drug screening using LC-MS/MS increased detection of a broader spectrum of compounds in our pediatric population. This approach has proven to be a reliable substitute for immunoassay-based drug screening as it offers superior specificity and sensitivity while obviating undesired confirmation cost and delay. Moreover, the LC-MS/MS provides a dynamic platform adaptable to changes in local patterns of drug supply and abuse.

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Development of an LC-MS/MS method for creatinine measurement in icteric subjects

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Background

Routine serum creatinine measurement is performed using automated chemistry analyzers that utilize either the Jaffé alkaline picrate or an enzymatic creatininase mechanism. Both of these methodologies are sensitive to bilirubin interference. In this study, we developed a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of creatinine in human serum samples that is insensitive to bilirubin interference.

Methods

Creatinine and its internal standard were analyzed using a Waters UPLC system (Milford, MA) coupled to an AB Sciex QTRAP 5500 mass spectrometer (Washington,

D.C.) in multiple reaction monitoring (MRM) mode. Chromatographic separation was performed using a Phenomenex normal phase column (Torrance, CA). The mobile phases consisted of 10 mM ammonium formate and 1.0% formic acid in water (phase A), and 1.0% formic acid in acetonitrile (phase B). Mobile phase B (90%) was ramped up to achieve 50% B over 2.0 minutes where it was maintained, followed by 1.30 minutes of re-equilibration in mobile phase A. Creatinine and its internal standard were detected by positive electrospray ionization with the following transitions: creatinine m/z 114→44 and internal standard m/z 117→47. Calibrators were created by spiking phosphate buffered saline with 1% bovine serum albumin (PBS w/ 1% BSA) with creatinine concentrations ranging from 0.05 to 5.0 mg/dL. Human serum samples were combined with labeled internal standard (creatinine-d3), and extracted using protein precipitation and dilution in a 96-well plate format. Method comparison studies were conducted on the Roche cobas 8000 (Indianapolis, IN) using the Roche Creatinine Plus Ver. 2 and Jaffé Gen. 2 reagent systems. Quality control (QC) samples were assayed on each day prior to testing. Bilirubin was evaluated indirectly by icterus measurement on the cobas 8000. Data reduction was performed using the Alternate Method Comparison Module on Data Innovations EP Evaluator® Version 9.4.0 software (South Burlington, VT).
Results
The LC-MS/MS method for creatinine measurement was linear from 0.05 to 5.0 mg/dL. Intra-day and inter-day precision was <3.3% and <10.8%, respectively. Accuracy by spike-and-recovery yielded recoveries from 88-100% for sixteen samples spanning the analytical measurement range. Specimen dilution was verified up to eight-fold. The reference interval was verified at 0.60-1.35 mg/dL for males, and 0.50-1.10 mg/dL for females. Stability was established for up to 7 days at ambient (20-25° C and refrigerated temperatures (2-8° C). Freeze-thaw stability was established for 5 cycles at both -70°C and -20°C. Specimen stability was verified up to 10 months at both -70°C and -20°C. No interference was observed for bilirubin concentrations exceeding 100 mg/dL. Creatinine measurement by LC-MS/MS demonstrated acceptable correlation to the automated enzymatic methodology (positive 1.2% average bias), but unacceptable correlation to the Jaffé methodology (positive 7.0% average bias). Both the Jaffé and enzymatic methodologies were more sensitive to bilirubin interference compared to LC-MS/MS.
Conclusions
LC-MS/MS provides an excellent alternative for the measurement of serum creatinine compared to routine automated methodologies that is insensitive to interference exceeding 100 mg/dL bilirubin. This methodology provides accurate creatinine determination in the context of hepatitis, HIV, and liver failure care patients.

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Validation of Prostate Cancer Biomarkers and Inflammation: A Proteomics Study

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Background: In this study serum protein profiles were analyzed in order to investigate possible confounding parameters in the discrimination between prostate cancer (PCa) and benign prostatic hyperplasia (BPH). **Methods:** Patients with clinical suspect of PCa and candidates for trans-rectal ultrasound guided prostate biopsy (TRUS) were enrolled. Histological specimens were examined in order to identify PCa, BPH and detect inflammation. Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-ToF-MS) and two-dimensional gel electrophoresis (2-DE) coupled with Liquid Chromatography-MS/MS (LC-MS/MS) were used to analyze immuno-depleted serum samples from patients with PCa and BPH. **Results:** The comparison between PCa (in the presence or absence of inflammation) and BPH (also in the presence or absence of inflammation) serum samples performed by SELDI-ToF-MS analysis, did not show differences in protein profiles. Differences became evident when the presence of inflammation was taken into consideration. When samples with histological sign of inflammation were excluded, 20 significantly different protein peaks were detected. Subsequent comparisons (PCa with inflammation vs PCa without inflammation, and BPH with inflammation vs BPH without inflammation) showed that 16 proteins appeared to be differently expressed in the presence of inflammation, while 4 protein peaks were not modified. With 2-DE analysis, comparing PCa without inflammation vs PCa with inflammation, and BPH without inflammation vs the same condition in the presence of inflammation, were identified 29 and 25 differentially expressed protein spots, respectively. Excluding samples with inflammation the comparison between PCa vs BPH showed 9 unique PCa proteins, 4 of which overlapped with those previously identified in the presence of inflammation, while other 2 were proteins, not identified in the previous comparisons. **Conclusions:** This study indicates that inflammation might be a confounding parameter during the search of candidate proteomic biomarkers of PCa. The results indicate that inflammation represents a significant confounding factor, hence, only a well-selected protein pattern should be considered as a potential biomarker of PCa.

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A Validated UPLC-MS/MS Method for Therapeutic Drug Monitoring of Sorafenib in Patients with Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) represents a global health problem and the incidence of this cancer in patient with cirrhosis is still increasing in several countries. There was no effective treatment available for patients diagnosed at advanced stage or who progressed into an advanced stage after other treatments failed. Sorafenib, an oral multikinase inhibitor with a potent antiangiogenic and proapoptotic activity, was approved with survival benefit. The concentration of sorafenib in the blood circulation is influenced by various physiological and pathophysiological effects in individual patients. A given dose of sorafenib can result in different plasma concentrations which may lead to sub-therapeutic drug exposure or increase adverse drug reactions at excessive plasma concentrations. The purpose for this study was to develop a method to quantitate sorafenib in plasma by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). **Methods:** The calibration curve samples were prepared by spiking drug free plasma with sorafenib tosylate. The stable isotope labeled sorafenib was used as an internal standard. After addition of the internal standard and protein precipitation, the supernatant was 10-fold diluted and injected into a chromatography system consisting of a UPLC BEH C18 (2.1 × 50 mm; particle size 1.7 µm) analytical column with gradient made of mobile phase (5 mM ammonium formate pH3.5 in water and acetonitrile containing 0.1% formic acid). The outlet of the column was connected to a triple quadrupole mass spectrometer with electrospray interface. Ions were detected in the positive multiple reaction monitoring mode. The concentration of analyte was calculated from the calibration curve and ion ratios between the analyte and the internal standard. **Results:** The analytical range was linear with a correlation coefficient of over 0.99 in the range of 19.5 - 10279.5 ng/mL. The imprecision for within-run was less than 1.3 % and between-run was less than 3.6 %. The accuracy was evaluated by spike recovery and the mean recovery was 101%-104%. This assay showed no ion suppression or enhancement and no carryover. The chromatography run time was 4.5 min. The assay was applied to quantitate sorafenib in plasma samples from 82 advanced HCC patients administrated different dose of sorafenib. Our data showed that the sorafenib concentrations vary markedly individual patients after equal dose, this therapeutic drug monitoring of sorafenib is essential to optimized sorafenib efficacy. **Conclusion:** A fast and accurate UPLC-MS/MS method to quantitated sorafenib was developed and successfully applied for routine therapeutic drug monitoring purposes in patients treated with sorafenib.

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A Novel Derivatization-Based LC-MS/MS Method with High Sensitivity for Quantitation of Cannabinoids in Breath Samples

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Background:
Nowadays cannabis use monitoring is in urgent demand for both public health and safety concerns. To facilitate the clinical study of a prototype breathalyzer for real-time cannabis use monitoring, a high-sensitivity LC-MS/MS method was needed to detect low-level cannabinoids in breath samples. We report a novel derivatization-based LC-MS/MS method with high sensitivity (pg/ml level) for quantitation of cannabinoids. The derivatization is based on azotization reaction which is compatible with water, methanol, and acetonitrile. It is not required to dry down samples before carrying out analyte derivatization. The assay has been applied to breath samples and the serum samples obtained from test subjects in a clinical study.
Methods:
A stock solution containing 5 cannabinoids THC, THCCOOH, 11-OH-THC, CBN, CBD was diluted in methanol or drug-free serum to prepare a calibrator series from 1000 pg/ml to 0.10 pg/ml. An IS (internal standard) solution was prepared in methanol. Derivatization assay (breath or serum): 100 µl of breath sample or supernatant of protein-precipitated serum sample was mixed with 2.5 µl IS solution. To derivatize

analytes, 20 µl of a diazonium solution was added and the sample was incubated at room temperature for 0.5 hr.
Non-derivatization assay (breath): 50 µl of each breath sample was mixed with 70 µl diluent with IS. Gradient elution was employed in HPLC separation. Quantitative analysis was carried out using MRM in triple-quadrupole mass spectrometer.
Results:
Derivatization assay for breath samples:
Imprecision was determined by running replicates of QC samples. Accuracy was determined by trueness of average results of QC samples. For all analytes, accuracy: 90.9% ~ 112.2% at QC L1 (5.0 pg/ml), 94.5% ~ 108.3% at QC L2 (50 pg/ml), 95.3% ~ 105.6% at QC L3 (200 pg/ml); imprecision (CV): 3.5% ~ 9.9% at QC L1, 1.0% ~ 4.6% at QC L2, 1.5% ~ 7.7% at QC L3. Linear range is from LOQ to 1000 pg/ml for all analytes. LOQ: THC 0.50 pg/ml (CV 5.8%, Accuracy 96.0%); THCCOOH 1.0 pg/ml (CV 7.5%, Accuracy 84.5%); 11-OH-THC 2.5 pg/ml (CV 15.1%, Accuracy 106.1%); CBN 0.10 pg/ml (CV 8.1%, Accuracy 103.6%); CBD 1.0 pg/ml (CV 13.5%, Accuracy 116.3%).
Derivatization assay for serum samples:
The derivatization method was applied to serum samples to demonstrate its broad applicability. Linear range is from LOQ to 1000 pg/ml. LOQ: THC 10 pg/ml (CV 9.7%, Accuracy 103.3%); 11-OH-THC 10 pg/ml (CV 8.4%, Accuracy 113.3%); CBN 2.5 pg/ml (CV 11.7%, Accuracy 114.4%). The derivatization assay is compared with the non-derivatization assay: THC level in 33 breath samples was analyzed using both assays, and excellent correlation was observed (Slope 1.12; R^2 0.997). In a clinical study, 9 test subjects were recruited, and timed collection of breath samples was implemented. Maximum THC level appeared at around 15 min after marijuana administration in all test subjects.
Conclusion:
The derivatization-based LC-MS/MS method has been proved to significantly enhance LC-MS/MS assay sensitivity. In a pilot clinical study of a breathalyzer-type point-of-care device, the derivatization method was applied to analyze breath samples which contain cannabinoids at pg/ml level. The derivatization method was also successfully applied to serum samples.

B-251

Water Soluble Vitamins, metabolites and derivatives determination by LC-MS in Blood for clinical research use

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Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC-MS/MS) is suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of the water soluble vitamins, metabolites and derivatives in blood and include- vitamin B1 (thiamine, thiamine pyrophosphate), vitamin B2 (riboflavin), vitamin B3 (nicotinic acid and nicotinamide), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxal 5-phosphate, pyridoxine, pyridoxal), vitamin B7 (biotin), folic acid, 5-methyltetrahydrofolate and vitamin B12 (cyanocobalamin). A simple sample preparation technique that involved a simple protein crash and liquid-liquid extraction were utilized along with a one (1D) dimensional liquid chromatographic configuration. The described method achieves the required sensitivity and is capable of determining the water soluble vitamins, derivatives and metabolites over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed for the quantitatively measurement of water soluble vitamins, derivatives and metabolites in blood. Method: A Thermo Scientific™ Quantis™ tandem mass spectrometer in positive Electrospray mode and a Thermo Scientific™ Dionex™ Vanquish™ Horizon HPLC system were initially utilized for this analysis. 200 µl of blood were used for the analysis of the water soluble vitamins, derivatives and metabolites in blood. Various columns were evaluated and an Thermo Scientific™ Accucore™ C18 100 x 2.1 mm, 1.5 µm with a water:methanol mixture containing 5 mM Ammonium Formate and 0.1% Formic Acid achieved baseline chromatographic separation for all the water soluble vitamins, derivatives and metabolites in serum in less than 8 minute run time. Quantitative analysis was performed using selective reaction monitoring (SRM) with transition pairs for each analyte and internal standard in positive mode and the precision and accuracy of the method was verified using pooled quality control materials and serum samples. Result: Good linearity and reproducibility were obtained with the concentration range of 1 to 50000 ng/ml for the respective water soluble vitamins, derivatives and metabolites in blood with a coefficient of determination >0.95 for the sample preparation technique and the LC-MS/MS configuration used. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.25 to 1 ng/ml and excellent reproducibility was observed for all compounds (CV < 10%). Conclusion: A sensitive, simple, specific and accurate liquid chromatography tandem mass spectrometry method was developed and verified for the simultaneous determination of water soluble vitamins, derivatives and metabolites in serum. The sample preparation technique is quick and easily applied for high throughput analysis.

B-252

Hydroxytyrosol stability in urine and synthetic urine matrices

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Background: The Primary Prevention of Cardiovascular Disease with a Mediterranean Diet (PREDIMED) study found a significant decrease in cardiovascular disease in a high risk population with dietary supplementation of either extra virgin olive oil (EVOO) or mixed nuts compared to a control group. EVOO contains elevated levels of phenolic antioxidants. Specifically, oleuropein and one of its metabolites, hydroxytyrosol, are of increasing scientific interest because they are thought to have antioxidant and anti-inflammatory benefits. In order to monitor compliance of EVOO intake, the levels of hydroxytyrosol have been monitored in urine. Unfortunately, studies have found that hydroxytyrosol has poor stability under certain conditions, such as in the presence of multivalent cations. We monitored the stability of hydroxytyrosol in urine and synthetic urine for four weeks to determine optimal storage conditions. We also employed an internal standard, D3-hydroxytyrosol, synthesized by the University of Minnesota's Institute for Therapeutics Discovery and Development.
Methods: Hydroxytyrosol was spiked into both urine and synthetic urine at concentrations of 20 ng/mL, 150 ng/mL, and 350 ng/mL and aliquoted into microcentrifuge tubes. The urine was pH 5.0, and synthetic urine was pH 7.5. Specimens were stored either native or spiked with acetic acid to a final concentration of 0.15M. Storage conditions ranged from room temperature, 4°C, -20°C, or -80°C for four weeks, and samples were tested on day 0, 1, 2, 7, 14, and 28. Specimens were processed by dilution with 150 µL of diluent (1M acetic acid, pH 4.5, with 10 mM oxalic acid) and addition of internal standard, D3-hydroxytyrosol. LC-MS/MS (QTRAP 6500, AB SCIEX) was used for analysis with a C18 Kinetex column (Phenomenex) and water/methanol as mobile phases.
Results: Recoveries observed for hydroxytyrosol in synthetic urine were the following: 0-30% for room temperature on Day 2, 6-25% for refrigerated at 4°C on Day 14, and 20-73% for frozen at -20 °C at Day 28. Only the synthetic urine samples stored at -80°C were found to have recoveries of 94-99% at Day 28. However, with the addition of 0.15M acetic acid, hydroxytyrosol was stabilized in all temperatures examined in this study with recoveries ranging from 94-106%. Hydroxytyrosol was stable in the urine specimen at 4°C, -20°C, or -80°C, regardless of whether acetic acid was present or not (recoveries of 89-108%). The addition of the isotopically labeled internal standard assisted in normalization of hydroxytyrosol degradation during processing of the urine/synthetic urine specimens.
Conclusion: Our data suggests hydroxytyrosol has better stability at lower pH. Degradation can be mitigated by either storing the specimens at -80°C or by the addition of acetic acid as a urine preservative. Additionally, we have improved our method by incorporating a deuterium labeled internal standard of hydroxytyrosol during preparation of specimens.

B-253

An LC-MS/MS assay with online extraction for measurement of testosterone in serum or plasma

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Background: Measurement of testosterone in circulation is important for evaluation of androgen status in men, women, and children. The very low concentrations of testosterone in children, females, and males with androgen suppression therapies necessitate the use of mass spectrometry (MS) based methods. We aimed to develop a simple and cost-effective LC-MS/MS assay for the quantification of testosterone at very low concentrations in serum or plasma.
Methods: This method was developed on a QTRAP 5500 LC-MS/MS system (AB SCIEX, Framingham, MA) coupled to a Shimadzu LC 30A HPLC. Serum or plasma sample (200 µL) was mixed with 400 µL acetonitrile containing $^{13}C_3$-testosterone as internal standard. The resulting mixture was spun at 4000 xg for 10 min. The supernatant (50µL) was injected into a C18 trap column in-line with the LC-MS/MS system. Statistics were calculated using Excel (Microsoft, Redmond WA, USA) and EP Evaluator Release 10 (Data Innovations, South Burlington, VT, USA).
Results: The linearity of the assay was assessed by serial dilution and found to be 10 to 10,000 pg/mL with analytical recovery from 82 to 87%. Precision was evaluated based on EP10-A3 protocol. For spiked plasma samples (N=30) with mean concentrations of 145 pg/mL, 4,554 pg/mL, and 9,032 pg/mL, the within run coefficients of variation (CV) were 2.8%, 3.5%, and 3.7%, respectively and the total CV was 4.1%, 3.7%, and 4.0%, respectively. No significant carryover was observed from samples

with concentrations up to 26,748 pg/mL. No significant interference was observed from androstenedione (5,000 pg/mL), dehydroepiandrosterone (80 ng/mL), epi-testosterone (10,000 pg/mL), and estriol (28 ng/mL). To assess the accuracy of the assay, we analyzed 40 patient samples offered by CDC Hormone Standardization Program (HoSt) Phase 1. These samples had assigned value ranging from 72.7 to 7,460 pg/mL. Deming regression of the results by this method and the assigned values showed a slope of 1.061, an intercept of -35.63 pg/mL and an R of 0.998, with a mean bias of 0.08 %. **Conclusion:** We have developed an accurate LC-MS/MS method for measuring serum/plasma testosterone with online sample extraction. This assay has been fully validated for clinical use.

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De Novo Amino Acid Sequencing of M-proteins by 21 Tesla FT-ICR MS Using Top-Down and Middle-Down MS/MS Techniques

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Background: The plasma cell disorders include multiple myeloma, AL amyloidosis, monoclonal gammopathy of undetermined significance, POEMS syndrome, and Waldenstrom's macroglobulinemia, which are characterized by a plasma cell clonal expansion. If there is clinical suspicion of one of these disorders, serum is tested for the presence of elevated levels of a monoclonal immunoglobulin (M-protein) secreted by clonal plasma cells. In contrast to typical cloning experiments, which are expensive, invasive and laborious, we report a non-invasive method for sequencing M-proteins from the blood which will enhance our ability to type the plasma cell disorders. **Methods:** Immunoglobulins were purified from patient serum with Melon Gel or camelid-derived nanobodies and digested with IdeS (FabRICATOR; Genovis). Disulfide bonds were then reduced with TCEP to produce antibody light, heavy Fd, and heavy Fc subunits (~25 kDa each), which were analyzed by reversed-phase LC-MS/MS. Mass spectra were acquired with our custom-built 21 T FT-ICR mass spectrometer, and MS/MS was performed with either electron transfer dissociation or collision-induced dissociation. Data were manually interpreted with Xcalibur 2.1 software (Thermo). Antibody isotype (constant region sequence) was determined by Xtract deconvolution, and fragments matched to the putative sequence by use of ProSight Lite (10 ppm fragment mass tolerance). Antibody variable region sequence characterization was performed by an in-house program, "AminoAcid Finder". **Results:** Nano-LC 21 T FT-ICR MS/MS was applied to analyze the M-proteins from AL amyloidosis patients sera (our group was blind to the M-proteins gene sequencing results). Based on our in-house developed top/middle-down *de novo* sequencing software, the M-protein light chain FR2-CDR2-FR3 was comprehensively characterized by MS/MS, and the assigned residues matched 100% to the corresponding gene sequence. The light chains were assigned to kappa KV1-33 germline sequence with seven amino acid mutations for the first sample and lambda LV3-21 germline sequence with five amino acid mutations for the second sample. The KV1-33 germline sequence is the most common kappa germline sequence identified in AL amyloidosis and is more likely to be associated with liver involvement, whereas the LV3-21 germline sequence is less commonly involved in AL renal patients. In another multiple myeloma sample, two M-protein light chains were detected with mass difference ~198 Da. MS/MS sequencing revealed that the two light chains belong to different germline sequences: kappa IGKV3-11 and kappa IGKV1-16. This example shows that our approach can simultaneously characterize more than one M-protein light chain, which is correlated with potential disease progression (e.g., malignant plasma cell mutation). Also, M-protein heavy chain glycoforms from multiple myeloma samples are well-known to differ from the glycoform profile in healthy human samples. **Conclusions:** We describe the first top/middle-down *de novo* sequencing of M-protein in serum with the advantages of ultrahigh mass accuracy and extensive sequence coverage. The results shown here serve as a blueprint for future characterization of endogenous M-protein in patients with a variety of immune system disorders. Work supported by the National Science Foundation through DMR-1157490 and DMR-1644779, and the State of Florida.

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Assessment of Mass Spectrometry Teaching in Pathology Residency

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Background:

Mass spectrometry (MS) has seen growing adoption in routine clinical laboratory testing in recent years. Indirect oversight of this testing is largely performed by medical directors boarded in anatomic and/or clinical pathology (AP/CP or CP), who may have limited familiarity with these instruments in the clinical setting. In an effort to train the next generation of pathologists to have adequate insight into this methodology, we conducted a needs assessment to determine the current availability and interest in mass spectrometry teaching in pathology residency from both program directors and residents. The findings of this study will be used to design curriculum to provide appropriate MS training for pathology residents.
Methods:
Two separate surveys for program directors and pathology residents were constructed and managed using REDCap electronic data tools hosted at University of California, San Francisco. The questions for program directors were designed to assess current and idealized teaching format. The questions for residents were designed to assess interest in MS training, perceived utility of MS in pathology practice, and idealized learning format. Surveys were sent to clinical chemistry program directors nationwide and pathology residents from the University of California, San Francisco.
Results:
Of the program director respondents (n=12), 66.7% currently have mass spectrometry teaching incorporated into resident teaching. The topics they felt residents should have the most exposure to include: principles of mass spectrometry (100.0%), clinical method validation (91.7%), and endocrine testing (75.0%). The perceived least important topics for resident teaching were proteomics (33.3%), metabolomics (25.0%), and mass spectrometry tissue imaging (25.0%). Of the resident respondents (n=21), 81.0% felt MS teaching would 'very important' to the future practice of clinical pathology. Additionally, 81.0% felt that it would be at least 'somewhat important' to the future practice of anatomic pathology. When asked if they felt MS would be important to their personal future practice of pathology, 52.7% noted it would be 'somewhat important', 23.8% thought it would be 'very important', 12.5% had no opinion, and 9.5% did not think it would be important. Residents voted that the most important topics to have exposure to were: principles of mass spectrometry (85.7%), microbial identification (61.9%), and toxicology (47.6%). The topics perceived least important were metabolomics (4.8%), endocrine testing (4.8%), and inborn errors of metabolism testing (0.0%). Both groups surveyed were asked how they would prefer MS curriculum to be taught. The majority of both groups (≥80%) agreed the topics should be didactic (rather than bench or elective research) and residents preferred dedicated mass spectrometry methodology lectures over incorporation into clinical topics.
Conclusion:
Of those surveyed, a majority of pathology programs have MS teaching in their residency, largely focusing on LC-MS/MS and clinical method validation. Furthermore, a large majority of pathology residents felt MS would be at least somewhat important to their future practice of pathology, if not very important. Both groups agreed teaching should be lecture-based, with residents preferring an emphasis on methodology over incorporation into clinical topics.

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Association of Plasma Metabolites with Brain MRI Measures in the Atherosclerosis Risk in Communities-Neurocognitive Study (ARIC-NCS)

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Background: Cerebral small vessel disease (SVD) contributes to dementia, cognitive and physical function decline. Our previous studies measured plasma metabolites by a targeted metabolomics method and established cross-sectional relations between higher levels of six plasma metabolites (two plasma phosphatidylcholines [PCs]: PC aa C36:5 and PC aa 36:6 and four sphingomyelins [SMs]: SM C26:0, SM[OH] C22:1, SM [OH] C22:2, SM [OH] C24:1) and favorable cognitive or physical function. The primary objective of this study was to conduct a hypothesis-driven analysis to investigate the relation of these 6 plasma metabolites with magnetic resonance imaging (MRI) features of cerebral SVD (small subcortical infarcts and white matter hyperintensities [WMH]) and brain atrophy. Both brain pathologies are common causes of adverse cognitive and physical function. Additionally, we explored the relation between 131 additional plasma metabolites with WMH or brain atrophy. **Methods:** This study included 238 older adults participating in the Atherosclerosis Risk in Communities-Neurocognitive Study (2011-13, mean age [standard deviation (SD)]: 77.5 [5.6], 56.7% women, 21.9% African Americans). Individual plasma metabolite concentrations were log-transformed and modeled in SD units. Multivariable

linear regression was used to assess the association of each metabolite in separate models with neuroimaging measures except lacunar infarcts; for lacunar infarcts, binary logistic regression was used. All models performed accounted for demographics, *APOE* genotype, cardiovascular risk factors, comorbidities, and use of medications. **Results:** We found that higher concentrations of plasma PC aa C36:5 and SM C26:0 had adverse associations with MRI features of cerebral SVD (odds ratio of 1.69 [95% confidence interval (CI): 1.01, 2.83] with lacunar infarct, and β of 0.16 mm^3 [0.02, 0.30] with log[WMH] volume) and total brain volume (β of -0.41 mm^3 [-0.75, -0.08] with deep grey white cortical volume), respectively; higher levels of 3 plasma SM (OH)s were associated with favorable features of cerebral SVD: β of -0.21 mm^3 [-0.33, -0.09] between SM (OH) C24:1 and log[WMH] volume, and β of 12.0 mm^3 [5.5, 18.6], 11.8 mm^3 [5.0, 18.6], 7.3 mm^3 [1.2, 13.5] between SM [OH] C22:1, SM [OH] C22:2, and SM [OH] C24:1 with total brain volume, respectively. In exploratory analyses, after Bonferroni correction ($p < 0.00038$), we found that while plasma metabolites such as plasma PC aa C38:6 and PC aa C40:6 had adverse associations with MRI features of cerebral SVD (odds ratio of 2.98 [1.68, 5.32] and 2.75 [1.65, 4.58] with lacunar infarct, respectively), other metabolites such as arginine, glutamine, and 3 plasmalogens (PC ae C40:4, PC ae C42:4, and PC ae C42:5) had favorable associations (β [95% CI] of -0.20 mm^3 [-0.30, -0.11], -0.22 mm^3 [-0.32, -0.11], -0.25 mm^3 [-0.38, -0.12], -0.29 mm^3 [-0.42, -0.16], and -0.25 mm^3 [-0.39, -0.12] with log[WMH] volume, respectively). **Conclusion:** Higher concentrations of several circulating metabolites (SM [OH]s) previously associated with neurocognitive and physical function endpoints independently correlate with more favorable measures of brain structure and neuroimaging abnormalities of SVDs.

B-257

Development and validation of cotinine in human urine by liquid chromatography electrospray ionization tandem mass spectrometry.

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Tobacco smoke is a widely recognized problem of public health due the risk factors leading to diseases as cancers and death worldwide. Determination of cotinine in urine is a good indicative of tobacco exposure. A simple, rapid and sensitive Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) was developed and validated for quantification of cotinine in human urine. In this process, 100 µL of urine were spiked with 25 µL of internal standard cotinine-d_3 and was treated with 200 µL of zinc sulfate 0.1M. Chromatographic separation was obtained using a UPLC System 1290 Infinity Agilent equipped with a Zorbax Eclipse Plus C18 RRHD column (2,1 X 50 mm 1,8 um) maintained at 35°C. Isocratic mobile phase consisting of methanol:water (98:2, v/v) with 0,1% of formic acid and 5 mM of ammonium formate at a flow rate of 300 µL.min^{-1}. The chromatographic run time obtained was 2.5 minutes. MS/MS detection was conducted a 6460 MS system (Agilent Technologies) by monitoring the fragmentation ions of 177→80 (*m/z*) and 177→98 (*m/z*) for cotinine and 180→80 (*m/z*) for cotinine-d_3. The linear range obtained for cotinine was 10.0-800.0 ng.mL^{-1} and dilution was validated for samples that exceed the curve in 4 times. Limit of detection (LOD) was 0.6 ng.mL^{-1} for cotinine. Within-day imprecision was less than 5.4% and between-day imprecision was less than 6.2 %. The medium range of recovery was between 94-109%. Cotinine was determinate with satisfactory sensitivity, precision, recovery and linearity. In conclusion, the method developed and validated has a quick and easy procedure for the measurement cotinine in human urine and can be applied to evaluation of the tobacco exposure.

B-258

Storage of urine specimens in POCT cups reduces concentrations of many drugs measured by confirmatory methods of urine drug testing.

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Background: Several pain management clinics use urine cups that contain point of care testing (POCT) urine drug strips to assess the presence of drugs in urine specimens. Some of the urine specimens that are received by clinical toxicology laboratory for confirmatory urine drug testing are in urine cups with POCT urine drug strips. We conducted this study to investigate the stability of drugs that are exposed to POCT urine drug strips before submitted for confirmatory urine drug testing. **Methods:** Drug free urine specimens in POCT urine drug test cups were spiked with common drugs that are tested in clinical toxicology laboratories. A dilute and shoot confirmatory liquid chromatography mass spectrometry method measured 87 drugs using Sciex 4500 instrument. The specimens were stored at room temperature and refrigerator conditions. The drugs concentration was measured after 30mins, 1hr, 2hrs, 5hrs, 6hrs, 24hrs, 48hrs and 96 hours when refrigerated and not measured until 24 hours when stored at room temperature. The concentration of drugs in urine specimen before exposure to POCT urine drug strips was used as the base line. Drug free urine spiked with the same drugs that were stored under the same condition in regular urine cups without POCT urine drug strips were used as a second control for the experiment. Alterations greater or lesser than 20% was defined as significant changes in drugs concentration. The drugs concentrations were selected at the cutoff concentrations. **Results:** Concentrations of amitriptyline, cyclobenzaprine, fentanyl, fluoxetine, flunitrazepam, nortriptyline, paroxetine and sertraline were significantly reduced within a range of 21-65% when urine specimen inside POCT cups were stored at room temperature for 24 hours. The spiked urine specimens that were stored in the same cups without POCT urine drug strips for 24 hours at room temperature did not show reduction in any of 87 tested drugs. The exposure of spiked urine to POCT urine drug strips for 24 hours in refrigerator significantly reduced the concentration of amitriptyline, cyclobenzaprine, paroxetine, propoxyphene, sertraline, duloxetine and buprenorphine. Exposure for 48 hours in refrigerator added, fentanyl, acetaminophen, duloxetine, haloperidol, atomoxetine, bupropion, desipramine and haloperidol, propoxyphene, fluoxetine, nortriptyline, dextromethorphan and doxepin to the list of affected drugs. Phencyclidine, methadone concentrations significantly reduced after 96 hours exposure of specimens to POCT urine drug strips in refrigerator. The reduction in concentration of drugs that were stored in POCT urine drug test cups in refrigerator varied between 22 to 45 %. When the spiked urine specimens were stored in refrigerator in urine cups without POCT urine drug strips for 96 hours, the concentration of all 87 measured drugs did not show any significant alterations. **Conclusion:** Exposure of urine specimens to POCT urine drug strips stored at room temperature or refrigerator reduces concentration of several drugs in urine drug testing performed by confirmatory liquid chromatography mass spectrometry method.

B-259

Determination of 7 Phosphatidylethanols in Blood using LCMS for clinical research use

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Background: Phosphatidylethanol (PEth) is an alcohol biomarker for alcohol use detection. PEth is a group of phospholipids containing 2 fatty acids of mainly palmitic and oleic acid (PEth 16:0/18:1 and 16:0/18:2) and a phosphoethanol. A simple, sensitive and specific LC-MS/MS analytical method was developed for the quantitation of the various phosphatidylethanols in blood using protein crash and a liquid-liquid extraction in blood sample preparation techniques. This easy method achieved good analyte recovery, post-extraction cleanliness and is capable of the sensitivities to quantitate the various phosphatidylethanols in blood over its dynamic range despite some challenging issues. Method: A Thermo Fisher TSQ Quantis tandem mass spectrometer in negative Electrospray mode and a Thermo Fisher Vanquish Horizon HPLC system were utilized. 100 ul of blood was used for the analysis of these compounds. Various columns were evaluated and initially a Thermo Fisher Accucore Phenyl-Hexyl 50 x 2.1 mm, 2.6 um with a water:acetonitrile/isopropanol mixture containing ammonium acetate achieved baseline chromatographic separation in less than 5 minute run time for PEth compounds. Quantitative analysis was performed using scheduled reaction monitoring (SRM) transition pairs for each analyte and internal standards of phospatidylpropanol and deuterated phosphatidylethanol were evaluated as to the most appropriate in negative mode. The accuracy of the method was verified using quality control materials and blood samples. Results: 7 various phosphatidylethanols were evaluated and it was determined that PEth 16:0/18:1 and 16:0/18:2 were the most prevalent in human blood. Good linearity and reproducibility were obtained with the concentration range from 0.05 to 500 umol/l for the various phosphatidylethanols with a coefficient of determination >0.995 for the sample preparation techniques employed. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to range initially from 0.01 and 0.025 umol/l. Excellent reproducibility was observed for both compounds (CV < 10%) and all techniques and configurations. Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of the various phosphatidylethanols in blood. The sample preparation techniques are quick and easily applied for high throughput analysis and the degree of phosphatidylethanol formation was further determined over time and correlates linearly with ethanol concentrations. Thus PEth can be used to determine long-term exposure to ethanol better than other ethanol biomarkers such as Ethyl glucuronide or Ethyl Sulfate.

B-260

An Easy-to-use Automated Solid-phase Extraction Method for Quantification of Serum Nicotine and Metabolites using LC-MS/MS

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Background: Tobacco use has a major impact on outcomes after medical procedures, such as solid organ transplant for both donors and recipients. LC-MS/MS analysis has been used for simultaneous quantification of nicotine, cotinine, and 3-OH-cotinine to provide accurate measurement of recent nicotine exposure. Such analysis usually involves with analytes extraction from the specimen, for example, SPE, which can be labor-intensive. We developed and validated an easy-to-use automated method for simultaneous extraction of nicotine, cotinine and 3OH-cotinine for subsequent quantification by LC-MS/MS. Some of the advantages of the relatively small bench-top instrument used in the study include user-friendly programming for multi-step extractions; and various size-options for both cartridge format and plate format. These features allow its use for different assays varied in test volumes and specimen volumes. **Methods:** Patient serum was mixed with deuterated internal standards prior to extraction. The automated SPE extraction was performed on a Biotage Extrahera System using Oasis® HLB cartridges in a 24-cartridge format, which has multiple steps including cartridge pre-treatment, sample loading, cartridge washing and analyte elution. The eluent was directly collected in LCMS vials for subsequent LC-MS/MS analysis, which was performed on a Thermo Vanquish UHPCL system coupled with Endura QQQ mass spectrometer. Separation of the three analytes was achieved on a C18 column using a linear gradient with a total LC time of 3.5 minutes for each injection. Two MRN transitions (one quantifier and one qualifier) were set for each target analyte with one MRN transition set for its corresponding internal standard. Simultaneous peak integration and quantitation for all three target analytes were achieved automatically using the pre-installed TraceFinder™ Software. **Results:** For each analyte, good linearity across the analytical measurable range was obtained. The limit of quantification (LoQ) was established at 2 ng/mL. Precisions of < 5% was obtained for both within-run and between-day studies. The accuracy was assessed by comparing patient results to the previously established LC-MS/MS method, with correlation efficiency of > 0.99 achieved for each analyte. Different degrees of matrix effects were observed for nicotine (70% mean recovery), conitine (95% mean recovery) and 3OH-cotinine (89% mean recovery). No ion suppression was noted for nicotine or cotinine with slight ion suppression noted for 3OH-cotinine. No interference was detected from nornicotine, norcotinie, or anabasine for each analyte. **Conclusion:** The validated assay provides an easy-to-use automated sample extraction for simultaneous measurement of nicotine, cotinine and 3OH-cotinine in patient serum using LC-MS/MS, which reduces the need for manual labor and offers good precisions. The programming and operation of the method are straightforward, which makes it easily adaptable for uses with different sample extractions prior to LC-MS/MS analysis.

B-261

Mucopolysaccharides Quantitation in Blood and Urine by LCMS for clinical research use

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Background: Mucopolysaccharides (MPS) or glycoaminoglycans (GAG's) are sulfated polysaccharides that contain repetitive disaccharide units attached to a protein core. The mucopolysaccharides are highly polar and act as a lubricant and metabolic disorders of abnormal accumulations of the MPS occur because of enzyme deficiencies. A simple, sensitive and specific LC/MS/MS analytical method was developed for the quantitation of Hyaluronic Acid (HA), Dermatan Sulfate (DS), Heparan Sulfate (HP), Keratan Sulfate (KS S1 and S2) and Chondroitin Sulfate (CS) and are enzymatically digested to disaccharides by the various MPS enzymes blood and using an acid digest and methanolysis in urine. This easy method achieved good analyte recovery, post-extraction cleanliness and is capable of the sensitivities to quantitate the MPS's in urine and blood over their dynamic range in both matrices despite some challenging issues. Method: A Thermo Fisher TSQ Quantis tandem mass spectrometer in positive and negative Electrospray mode and a Thermo Fisher Vanquish Horizon HPLC system were utilized. 100 ul of urine and blood were used for the analysis of these compounds. Various columns were evaluated and initially a Thermo Fisher Accucore C18 100 x 2.1 mm, 2.6 um with a water:acetonitrile mixture containing 5mM Ammonium Acetate achieved baseline chromatographic separation in less than 8 minute run time for all compounds. Quantitative analysis was performed using scheduled reaction monitoring (SRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using quality control materials and blood and urine samples. Results: Good linearity and reproducibility were obtained with the concentration range from 1 to 2000 nmol/l for the various MPS's with a coefficient of determination >0.995 for both sample preparation. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to range initially from 0.2 and 0.5 nmol/l. Excellent reproducibility was observed for both compounds (CV < 10%) and all techniques and configurations. Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of MPS in urine and blood. The sample preparation techniques are quick and easily applied for high throughput analysis and included enzymatic and acid digestion in blood and urine. The identification of MPS-I, II, III, IV and V could easily be achieved.

B-262

A Clinical Research LC-MS/MS Method for the Measurement of Serum Estrogens

R. M. Wardle, L. J. Calton. *Waters Corporation, Wilmslow, United Kingdom*

Background: The two major biologically active estrogens in non-pregnant humans are 17β-estradiol (E2) and estrone (E1). E2 is produced primarily in the ovaries and testes by the aromatization of testosterone, whereas, E1 is primarily derived from androstenedione. E2 can be metabolized to E1 and conversion of E1 to E2 is also possible, making the measurement of both compounds desirable. Some immunoassay techniques lack analytical sensitivity and selectivity for E2, whilst published LC-MS/MS methods often use large sample volumes with complex sample preparation, including derivatization to reach the analytical sensitivity and selectivity requirements. Here we describe a simple method for the measurement of E2 and E1 by LC-MS/MS for clinical research. **Methods:** Samples (250µL) were spiked with $^{13}C_3$ labeled internal standards and a liquid/liquid extraction performed using a mixture of hexane and ethyl acetate. Following centrifugation, the top layer was transferred to glass vials, evaporated to dryness and reconstituted using methanol/water prior to analysis. Chromatographic separation was achieved, in less than 5 minutes, using a Waters® CORTECS® Phenyl column (2.1 x 50mm, 2.7µm) with a water/methanol/acetonitrile/ammonium fluoride gradient on the Waters ACQUITY UPLC® I-Class system. E2 and E1 were detected using electrospray negative ionization on a Waters XEVO® TQ-XS mass spectrometer. In-house calibrators (2-1000pg/mL) and quality controls (3, 30 and 175pg/mL) containing both E2 and E1 were prepared using reference material from Cerilliant (Round Rock, TX) and MSG4000 stripped serum from Golden West Biologicals (Temecula, CA). **Results:** Total precision and repeatability assessments for E2 and E1 in samples spiked across the calibration range 2–1000pg/mL were ≤8.4% CV, except for the lowest calibrator level at 2pg/mL (≤12.5% CV). Analytical sensitivity of the method allows for E2 and E1 measurement at 2pg/mL, determined over 5 occasions (n=5 extractions), where the precision was ≤20%CV and signal to noise ratio (ptp) was >10:1. Accuracy of the method for E2 was determined by analyzing 40 Phase 1 samples from the CDC Hormone Standardization Program (Atlanta, GA); good agreement was observed with a Deming fit of y=1.14x – 1.85 and a mean bias of ≤10.9 % (Altman-Bland). The method was shown to be linear over the range 0.43–1108pg/mL for E2 and 0.65–1113pg/mL for E1, with no significant carryover up to 2000pg/mL. Quantitative matrix factor was evaluated using six individual serum donors. Matrix factors for both analyte peak area and analyte:internal standard response were calculated. Ion enhancement was observed but compensated for using the internal standard (E2 range -13.2% to 2.0% and E1 range -14.7% to 9.1%). Endogenous and exogenous interferences were assessed and the mean recovery observed was between 85–115%. **Conclusions:** A simple LC-MS/MS method has been developed for the measurement of serum estradiol and estrone. This clinical research method demonstrates good precision, linearity, analytical sensitivity and accuracy. For Research Use Only, Not for use in diagnostic procedures.

B-263

Targeting Production of a Fast-Forming Proteotypic Peptide for Rapid Quantification of Apolipoprotein A1 in Plasma by LC-MS/MS

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Background: Apolipoprotein A1 (apoA1) is the major protein component of high-density lipoprotein particles in plasma, comprising up to 70% of the total protein mass. Clinically, concentrations of apoA1 in serum or plasma are used in risk assessment of cardiovascular events. Clinical laboratories commonly use nephelometric or turbido-

metric methods to measure apoA1, which can be costly, require a relatively large volume of sample (e.g. upwards of 150 µL of serum or plasma) and prone to interferences common to immunoassays (i.e. lipemia, cross-reactivity, etc.). An alternative methodology, liquid chromatography–tandem mass spectrometry (LC-MS/MS) has been previously applied to the measurement of apoA1; however, uptake of these methods for routine clinical testing has not been realized due to complex and time-intensive sample preparation workflows. Toward the design of an assay suitable for implementation in a clinical laboratory, we simplified the sample preparation workflow including eliminating reduction and alkylation, and using only additive steps. By optimizing digestion conditions and monitoring digestion profiles of nine different apoA1 proteotypic peptides, we identified a peptide (THLAPYSDELR, residues 185-195) demonstrating rapid and stable digestion kinetics. Herein we describe the design and validation of a simple and rapid quantitative apoA1 LC-MS/MS assay targeting this fast-forming peptide. **Methods:** For the external calibrators, the peptide sequence THLAPYSDELR (unlabeled) was synthesized with concentration assigned by HPLC and amino acid analysis. We used a C-terminal $^{13}C/^{15}N$-Arg labeled peptide as the internal standard (IS), and pooled human EDTA plasma as quality controls (QC_1 = 1.00 g/L; QC_2 = 1.53 g/L). Samples were diluted in phosphate-buffered saline and added to a 50 mmol/L ammonium bicarbonate buffer containing the IS. Samples were mixed briefly and denatured by heating at 99 °C for 10 min. Samples were then cooled to room temperature and N-tosyl-L-phenylalanine chloromethyl ketone treated trypsin was added for digestion. Samples were incubated at 37 °C for 20 min. Note that all steps are additive and no centrifugation or separation is required. The method validation followed Clinical & Laboratory Standards Institute guidelines, which, briefly, includes assessment of sensitivity, precision, accuracy, linearity, interferences, and stability. **Results:** Using our rapid digest protocol requiring a brief 20 min digestion, proteotypic peptide THLAPYSDELR was selected for quantitation of apoA1. The amino acid analysis-assigned calibrators ranged from 0.005 – 0.300 g/L. With plasma specimens subjected to a 10-fold dilution, the clinically reportable range was 0.05 – 3.00 g/L. QCs were stable through at least 4 freeze-thaw cycles and could be left at room temperature for at least 4 days. Method comparison (n = 40) of our LC-MS/MS method to the Siemens BNII Chemistry system immunonephelometry apoA1 assay revealed the following linear regression: LC-MS/MS = 0.70 × immunonephelometry – 0.09, R^2 = 0.9148, CI_{slope}: 0.63,0.78. The intra-assay precision was 3.30% (QC_1) and 6.70% (QC_2) and the inter-assay precision study is ongoing. **Conclusions:** By streamlining sample preparation and optimizing conditions of denaturation and digestion, we were able to develop a simple and rapid LC-MS/MS method for quantitation of apoA1 in human plasma.

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A rapid and simplified LC-MS/MS workflow for the analysis of pain management drugs for clinical research.

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Background: Comprehensive pain management and drug panels for clinical research analysis often include such commonly used substances such as opioids, benzodiazepines and stimulants. Other classes of compounds can include muscle relaxants, anti-epileptic drugs such as gabapentin, synthetic cathinones ("bath salts") and other substances. Often, multiple methods are used to obtain a comprehensive view of the various drug classes. Key workflow considerations include the use of fewer, more comprehensive analyte panels and rapid sample preparation and analytical techniques, all of which must be balanced against the need for sample integrity and data quality. The objective of this study was to develop a comprehensive LC-MS/MS analysis strategy for a large drug panel (80 compounds from 22 drug classes) using a simplified solid phase extraction (SPE) protocol that incorporates in-well hydrolysis and pre-treatment of the urine sample. **Methods:** Calibration curves were prepared by spiking the compounds into urine covering the appropriate measurement range for each compound, from 2-200 ng/mL for 6-MAM and fentanyl, to 25-2,500 ng/mL for many opiates and amines. Urine samples (spiked calibrators, QCs and independent QC material from UTAK) were extracted using mixed-mode cation exchange polymeric SPE plates. All pre-treatment steps were conducted within the SPE plate wells. Analytes were extracted using a modified procedure designed to extract all components in a single protocol. LC-MS/MS analysis was conducted using a Waters ACQUITY I-Class UPLC system coupled to a Xevo TQ-S micro mass spectrometer under reversed-phase conditions. **Results:** The method was evaluated for linearity, precision, accuracy (recovery), extraction efficiency and matrix effects. All analytes eluted within 3.1 minutes while maintaining baseline separation of all isobaric compounds. Calibrator linearity was assessed and QC results were accurate and precise for all compounds over the measurement range. Precision performance over five days was acceptable both within and between runs (%CV<12%). Accuracy was assessed using commercially available control materials from UTAK (mean bias < 6.2 %) and all spiked QC sample determined concentrations ranged from -7.8% to 16.2% bias of the target values at the lowest QC level and within ±10% bias of target values at all other levels, with an overall mean bias of <3%. Extraction efficiencies were high and consistent for all compounds, averaging >80% with %RSDs under 15% for all analytes. Internal standard corrected matrix effects were less than 20% for all but 3 compounds, with %CVs <20%. **Conclusion:** This method enables the rapid extraction and analysis of a diverse panel of drugs for clinical research. A single, rapid SPE method is used to extract 80 compounds with high efficiency. The use of 96-well plates, combined with in-well sample pre-treatment eliminates sample transfer steps, minimizing the risk of cross-contamination or sample transfer errors. The combination of sample preparation, chromatography and tandem MS analysis results in a complete and comprehensive workflow. For Research Use Only, Not for use in diagnostic procedures

B-265

Evaluation of the Shimadzu CLAM 2000 fully integrated, automated sample preparation system for LCMS in the routine clinical laboratory.

L. Bachmann. *Virginia Commonwealth University, Richmond, VA*

Background: Lack of availability of fully automated LC-MS/MS systems has significantly impeded adoption of the technology into the routine clinical laboratory. We evaluated the analytical performance and workflow of the Shimadzu Clinical Laboratory Automation Module 2000 (CLAM-2000)/Nexera XR LCMS-8050 for analysis of total 25-OH Vitamin D (25-OHD) in a clinical laboratory setting. Because many methods for 25-OHD do not resolve the 3-epi isomer of 25-OHD3 (which is thought to be an inactive form of vitamin D), we specifically developed the CLAM 2000/Nexera XR LCMS-8050 method to enable quantification of 3-epi 25OHD3. **Methods:** The method was calibrated using commercially available Chromsystems 6PLUS1 calibrators for 25-OHD3 and 25-OHD2. 3-epi 25-OHD3 calibrators were prepared by spiking 3-epi 25-OHD3 into stripped serum to final concentrations of 0, 5, 10, 20, and 40 ng/mL. Sample preparation was fully automated using the Shimadzu CLAM-2000 module. Briefly, a 1% Zinc Sulfate/95% MeOH mixture containing internal standard for 25-OHD3, 25-OHD2 and 3-epi 25-OHD3 was added to 30µL of patient serum. After automated mixing and onboard incubation for 15 min, the precipitated solution was filtered through a 0.45µm PTFE membrane by vacuum filtration. 15 µL of the filtrate was automatically injected and analyzed using the Nexera XR LCMS-8050 system. A MeOH/dH20 mobile phase gradient was used to obtain separation on a 2.7µm Raptor FluoroPhenyl LC Column (Restek) with guard column. MS analysis was performed using APCI in positive ion mode. Method validation experiments appropriate for a Laboratory Developed Test (LDT) were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** The method was linear from 2 ng/mL - 150 ng/mL for 25-OHD3 and 25-OHD2, and 2 ng/mL - 40 ng/mL for 3-epi 25-OHD3. For between-run imprecision, coefficients of variation were < 6% at 5.2 - 60.9 ng/mL and < 15% at 2 ng/mL. Patient sample comparisons were performed using a routine LC-MS/MS method used in our clinical laboratory that employs manual preanalytical processing. The CLAM 2000/Nexera XR LCMS-8050 method agreed with the routine laboratory method and exhibited an average systematic error of < 6%. Bias vs. National Institutes of Standards and Technology (NIST) SRM 972a Vitamin D reference materials was ≤ 10.9% for all four levels of materials. 13/94 patient samples had 3-epi 25-OHD3 ≥ 2ng/mL, however the concentrations were not clinically significant. Use of the CLAM-2000 Nexera XR LCMS-8050 system saved approximately 80 minutes of labor vs. our routine laboratory method. **Conclusions:** The CLAM-2000 Nexera XR LCMS-8050 system exhibited similar analytical performance to our validated laboratory method for total 25-OHD, decreased labor requirements and improved workflow efficiency for LC-MS/MS testing in the clinical laboratory. The method also enabled quantification of 3-epi 25-OHD.

Wednesday, August 1, 2018

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

Molecular Pathology/Probes

B-266

Detection of possible new cytogenetic alteration by arrayCGH related to dysmorphia in the hands

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Background: Comparative Genomic Hybridization Arrays (CGHarrays) are technologies that allow detecting losses or gains of genetic material and unbalanced rearrangements throughout the genome with high sensitivity, speed and resolution. **Methods:** A 13 year old patient with a possible dysmorphic syndrome is studied for presenting disorder of the joint movement of the fingers of both hands that hinder movement. As a family history he emphasizes that his father has similar alterations in the fingers. The arrayCGH is carried out with the PerkinElmer platform which has an average resolution of 20kb and of 10kb in the regions of interest. The Genoglyphix software is used to interpret the results. **Results:** A deletion of 1.98 Megabases is detected in the chromosomal region 1q41 The study is also carried out in his father for presenting also the pathology detecting the same alteration found in his son. **Conclusion:** The deletion found has not been associated to date pathology or polymorphism, so it could be considered as of clinical significance uncertain, but when detected also in the parent with a high probability could be cataloged this alteration as the Cause of pathology. With this technique can be revealed alterations that can be cause of mental retardation, intellectual disability, dismorfias and/or malformations, which represent a major public health problem. Thanks to the arrayCGH, the proportion of cases whose etiology is unknown can be minimised.

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A case of 4p deletion syndrome inherited from an affected parent without phenotypic features

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Background: Partial deletions of the short arm of chromosome 4 is associated with a clinically recognizable syndrome. Wolf-Hirschhorn syndrome (WHS) is a rare chromosomal disorder caused by terminal deletion of the short arm of chromosome 4. About 50%-60% of individuals with WHS have a *de novo* deletion and about 40%-45% have an unbalanced translocation with a deletion of 4p. These unbalanced translocations may be *de novo* or inherited from a parent with a balanced rearrangement. The clinical picture includes facial anomalies, growth retardation, mental retardation, seizures and defects in the brain, heart and palate. It is reported that the sizes of 4p deletions are variable and the clinical findings are different from classic WHS according to the deleted portion. The critical region for WHS is located on the terminal part of the chromosome 4p, with a length of approximately 1-5 Mb. The severity of the clinical presentation is variable depending on the haploinsufficiency of genes in a deleted region. **Objective:** To report a case of a maternally inherited 4p deletion from asymptomatic woman analyzed by G-banding karyotype by array-based comparative genomic hybridization (array-CGH). **Case report:** We report four-year-old boy with severe hypotonia, hypospadias, psychomotor development delay, hypertelorism, cardiac anomalies, seizures and defects in the brain. He was born to nonconsanguineous and asymptomatic parents, with no history of obortions. G-band chromosome analysis revealed the karyotype 46, XY, del(4)(p16). His father had a normal karyotype (46,XY), but the maternal chromosomal analysis revealed the 46,XX,add(4)(p15.2) karyotype. The proband's array-CGH analysis revealed an 8.6 Mb pathogenic deletion in the chromosome 4p16.3p16.1 [arr[hg19] 4p16.3p16.1(85,743-8,702,376)x1] encompassing more than 80 genes, including the WHS critical region. Maternal CGH array analysis revealed an interstitial 5.5 Mb deletion in the chromosome 4p16.3p16.1. This deletion includes several OMIM genes such as HTT, DOK7, ADRA2C, EVC, EVC2, but these genes alone were not related to any phenotype, except for the MSX1 and WFS1 genes. The WFS1 gene is relates to Wolfram Syndrome and non-syndromic sensorineural hearing loss, whereas the haploinsufficiency of the MSX1 gene is associated with selective dental agenesis. Thus, a smaller deletion was detected in this case, excluding the WHS critical region. The recurrence of WHS in the child was not due to a parental balanced translocation, but it was due to a different mechanism, the meiotic amplification of a smaller deletion present in the mother, most likely. This case highlights the importance of an integrated approach to cytogenetic analysis and array-CGH analysis.

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Expression of Tim-3 in PBMCs and Placental Tissue in Unexplained Recurrent Spontaneous Abortion

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Background: The expression of T-cell immunoglobulin domain, mucin domain-3 (Tim-3) in unexplained recurrent spontaneous abortion (URSA) was investigated. **Methods:** im-3 mRNA expression in peripheral blood mononuclear cells (PBMCs) of URSA and control groups was assayed by fluorescent quantitative real-time polymerase chain reaction. Tim-3 protein expression intensity and localization in placental villi and uterine decidua were determined using immunohistochemical assay. **Results:** Tim-3 mRNA expression in PBMCs was significantly higher in URSA than in normal controls (1.32 ± 0.13 vs. 1.20 ± 0.06, $P < 0.01$). Tim-3 was expressed in placental tissue from both URSA patients and normal pregnant females (controls); however, the expression intensity was higher in the URSA group ($P < 0.05$). **Conclusion:** Increased Tim-3 expression in PBMCs may affect maternal-fetal immune tolerance and facilitate pathogenesis of URSA.

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distribution of the genotype of interleukin-28b sr12979860 in patients with chronic hepatitis c studied in our hospital area

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Background: According to data from the World Health Organization, infection with the hepatitis C virus represents a serious global health problem, since it is estimated that it affects approximately 180 million people, of which 70-80% will develop an infection chronicle. In these patients with chronic infection, the polymorphism rs12979860 of IL28B has been described as an important predictor of response to treatment with pegylated interferon alfa (INF alpha) and ribavirin. Thus, patients carrying the CC polymorphism have percentages higher than 80% sustained viral response (SVR), while CT and TT patients have SVR rates of less than 50%. The aim of this study is to determine the distribution and frequency of the different polymorphisms of IL28B rs 12979860 in patients with chronic HCV infection studied in our sanitary area. **Methods:** A descriptive, retrospective study was conducted to determine the distribution of IL28B rs 12979860 in patients with chronic HCV studied in our health area for a period of 6 years (January 2011 to January 2017). The IL28 polymorphism determination was performed by real-time polymerase chain reaction (PCR), using specific fluorescent probes for the C and T alleles of SPN rs 12979860. **Results:** From January 2011 to January 2017 2465 patients were analyzed. Males represented 71% of the studied population with an average age of 54 years with a range of 29 to 79. Women accounted for 29% with an average age of 51 years and a range of 31 to 83 years. The analysis of the distribution of IL28B polymorphism results showed that 36.31% (895/2465) were homozygous CC, 49.86% (1229/2465) were heterozygous CT, and 13.83% (341/2465) were homozygous TT. **Conclusions:** -There is a high percentage (63.69%) of patients with CT and TT polymorphism that are associated with low rates of sustained viral response to treatment with IFN alpha and rivabirine. -As in the literature consulted, the CC genotype was found in 36.31% of these patients. -The typing of the IL28B polymorphism allows evaluating the probability of response to INF plus ribavirin in patients with chronic HCV allowing the adjustment thereof over time by following the individual requirements increasing it in individuals with CC genotype and looking for new therapies in the CT and TT genotypes.

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ABL1 transcripts levels in peripheral blood mononuclear cells of healthy individuals: comparison of three automated nucleic acid extraction instruments.

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Background: The ABL1 mRNA is widely used as the reference gene for quantification of leukemic fusion transcripts, such as BCR-ABL and PML-RARA. The ABL1 quantification is used to ensure the analytical assay sensitivity, which is essential for the minimal residual disease monitoring along and after therapy. A sensitive BCR-ABL and PML-RARA RT-qPCR process should quantify 10^5 copies of ABL transcripts in every reaction. Here, we compared three automated systems for nucleic acids (NA) extraction from peripheral blood mononuclear cells (PBMC) to evaluate the ABL1 levels returned by each system. **Methods**: PBMC of healthy volunteers (60 women and 26 men, average age 42.21±17.59 years) was isolated from EDTA-whole blood samples (4 mL, n=86). Residual red blood cells were removed using ammonium chloride-based buffer (BD Biosciences). PBMC was resuspended in the initial sample volume required for each equipment. PBMC total nucleic acids (or RNA) were extracted on QIAcube (Qiagen) using RNeasy kit (PBMC resuspended in 600 uL, RNA elution in 100 uL) (n=24); on MagNA Pure 96 (Roche) using DNA/Viral large volume kit (PBMC resuspended in 1mL, NA elution in 100uL) (n=32); or on easyMAG (Biomerieux) (PBMC resuspended in 1mL, NA elution in 65 uL) (n=30). ABL1 mRNA was amplified by one-step RT-qPCR using Europe Against Cancer primers/probes and QuantiNova probe master mix (with DNase) (Qiagen) on Light Cycler 480 II or 480z (Roche). Quantification standard curves were constructed with ssDNA oligos corresponding to the ABL1 primers molecular target. Medians ABL1 copy number per reaction were compared using Kruskal-Wallis test followed by Dunn's multiple comparisons test. The percentage of samples with higher than 10^5 copies per reaction of ABL1 transcripts were compared by Chi-squared test followed by Marascuilo procedure (Multiple comparisons). **Results**: The median (Max-Min) of ABL1 copies per reaction was $7.5x10^4$ ($1.5x10^5$-$2.4x10^4$) for QIAcube, $1.3x10^5$ ($1.7x10^5$-$5.8x10^4$) for MagNAPure and $1.1x10^5$ ($2.6x10^5$-$7.6x10^3$) for easyMAG. The median copy number obtained using easyMAG and MagNAPure were similar (p=0.40). However, both were different from QIAcube results (p=0.0001 and p=0.0011, respectively). The percentage of samples that achieved 10^5 copies of ABL transcripts per reaction was 25% for Qiacube (p<0.0001 *versus* easyMAG and p=0.0083 *versus* MagNAPure), 85% for MagNAPure (P=0.27 *versus* easyMag) and 97% for easyMAG. **Conclusion**: EasyMAG and MagNAPure extracted a higher median copy number of ABL transcripts per reaction compared to QIAcube. Additionally, a higher percentage of samples reached the limit of 10^5 copies of ABL transcripts using the prior instruments. These results suggest that easyMAG and MagNAPure can consistently provide better sensitivity for the assessment for the minimal residual disease of leukemic fusion transcripts. The limitation of this study is its small number of tested samples.

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Frequency distribution of p210, p190, and p230 fusions transcripts on a BCR-ABL1 laboratory routine

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Background: Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells present in 90% of CML patients that originate from the reciprocal translocation of ABL1 sequences from chromosome 9 to BCR sequences on chromosome 22. Around 95% of the CML patients are reported with e14a2/e13a2 (p210) BCR-ABL1. A very few patients had the BCR-ABL1 fusion e1a2 (p190). The BCR-ABL1 fusion e19a3 (p230) is even rarer. Co-expression of p210 and p190 in patients with classical CML or ALL have been described. Recently our group validated a simplified workflow for simultaneous detection/quantification of BCR-ABL1 e14a2/e13a2 (p210), e1a2 (p190), and e19a2 (p230) fusions by one-step RT-qPCR using ΔΔCq method. Here, we to describe the assay's frequency of results from August 15, 2017, to February 19, 2018. **Methods**: EDTA-whole blood (8 mL) or bone marrow samples (4mL) of 126 individuals (57 female, 69 male) were processed and tested for BCR-ABL1 e14a2/e13a2 (p210), e1a2 (p190) and 19a2 (p230) mRNAs. ABL1 was used as reference gene. Nucleic acids were extracted by using Magna 96 (Roche). We used the one-step RT-qPCR QuantiNova Probe master mix (Qiagen) on the routine assays. Primers and probes used were described by Gabert et al. 2003 (EAC) and by Pane et al. 1996. BCR-ABL1 and ABL RNAs were co-amplified in a multiplex one-step RT-qPCR reaction performed in the Roche LightCycler Z480 or 480II. P210 was calibrated to International Scale (IS) with a calibrator panel (Asuragen). Each fusion reaction contained Three levels of quality control material were used in each fusion reaction. The molecular response (MR) scoring used on follow-up patients was described by Cross et al. 2015. Descriptive statistics were determined for all the datasets. **Results**: BCR-ABL1 mRNAs assay was performed on 125 samples and 54 (43.2%) tested positive (26 males and 28 female). The median age of positive patients was 52 (range 8-82 years). Twenty positive patients (37.03%) had high expression levels of p210, 12 (22.22%) co-expressed p190 at low levels. One positive patient (1.85%) had higher expression levels of p230 and co-expressed p190 at a low level. Among the patients with low expression levels of BCR-ABL1 transcripts (<10%), 32 (59.25%) had p210, 1 (1.85%) p230 and 1 (1.85%) p190. Among the p210 with low levels, 9 (28.12%) were at MR1 (1-10%), 11 (34.37%) were at MR2(0.1-1%), 11 (34.37%) were at MR3 (0.01-0.1%), and 1 (3.12%) was at MR4 (0.001-0.01%). **Conclusion**: Co-expression of more than one BCR-ABL1 fusion was common in samples with high expression levels of p210 and p230, which is in agreement with literature data and may be explained by the alternative splicing. It is also important to point out that clinical laboratories usually do not test p230 BCR-ABL1 and we found the presence of two p230 positive patients and only one p190 positive patient, suggesting the importance of testing for these transcripts on the BCR-ABL1 fusions detection routine. The main drawback was the low number of samples tested.

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Whole-exome sequencing as first-tier testing approach for identification of the causal mutations in hereditary spherocytosis candidate genes and the use of non-sanger-based methods for validation of the findings

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Background: Hereditary spherocytosis (HS) is a common form of inherited hemolytic anemia characterized by hemolysis, jaundice, splenomegaly, and gallstones. The condition commonly exhibits an autosomal dominant pattern of inheritance. Causal mutations in at least five genes have been reported so far (SPTA1, SPTB, ANK1, SLC4A1, and EPB42). In this study, we aimed to investigate the five HS genes performing whole-exome sequencing (WES) to identify the causal mutations in a cohort of subjects with the condition. A secondary aim, we tested if non-sanger-based methods, such as ARMS-qPCR (for SNV) and capillary electrophoresis (for INDELs), can be used to validate the next-generation sequencing findings. **Methods:** The studied cohort comprises 16 patients (8 males) with HS diagnosis. There was one pair of siblings, one mother-daughter pair, one mother and two sons, and nine unrelated subjects. DNA was extracted from whole blood using MagNA Pure 96 (Roche), quantitated using Qubit (Thermofisher), and sized and qualified by gel electrophoresis. Targeted resequencing library was captured using SureSelect All exon V5-post kit (Agilent Technologies). Genomic libraries were quantified and validated using TapeStation (Agilent Technologies) and sequenced as paired-end 150 bp reads on the NextSeq 500 sequencer (Illumina). Sequence reads were aligned to human reference genome 19 using the BWA aligner. Duplicates were removed using Picard and variant calling was performed using the GATK Unified Genotyper (both from Broad Institute). SNV and INDELs were annotated using BaseSpace Variant Interpreter (Illumina). Variant lists were filtered based on having a minor allele frequency of less than 0.01 on population frequency databases [1000 Genome Project, NHLBI exome sequencing project and exome aggregation consortium (ExAc)]. Mutations that had read depth (coverage) less than 8, quality score less than 20, or resulted in synonymous amino acid changes were excluded. Sanger sequencing and ARMS-qPCR or capillary electrophoresis were compared for the validation of NGS results. **Results:** Pathogenic mutations were identified in 14 out of 16 (87.5%) studied subjects (all in heterozygosis). Six patients had a mutation in the SPTB gene [p.(Arg1423Ter) occurring twice and p.(Gln804Ter) occurring three times, because the subjects were relatives], six patients had a mutation in the ANK1 gene [p.(Arg319Ter), p.(Gln1806Ter), p.(Glu101Ter), p.(Gly122Arg), c.4227+1G>A and c.5518-1G>A], one patient had a mutation in the SPTA1 gene [p.(Tyr2305Ter)]. All of them have not been previously described and appear to be novel findings. One patient had both SLC4A1 [p.(Trp848Arg)] and SPTA1 [p.(Glu110Val)] mutations, which were found in the ExAc population database at very low frequencies (MAF of 0.000015 and 0.00022, respectively). All identified mutations were confirmed by Sanger sequencing and by ARMS-qPCR or capillary electrophoresis. Two patients (siblings) had no mutation in the five HS genes according to the used filtering criteria and will proceed to all hereditary anemia genes sequence inspection (followed by WES inspection if none causal mutation is found). **Conclusions:** The use of whole-exome sequencing as the first-tier testing approach was effective in identifying the causal mutations of HS in the studied cohort (87.5%

of the volunteers had the causal mutation identified) and non-sanger-based methods could validate the NGS results.

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Agreement between UV absorbance, automated microfluidic electrophoresis and qPCR methods for DNA quantification

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Background: DNA quantification is a critical step for next-generation sequencing (NGS) aiming to investigate genomic phenomena (e.g., mutations in cancer and diseases). Accurate quantification of the DNA starting material ensures consistent and reproducible results from NGS library preparation. Here, we aim to compare three methods with different methodologies for NGS input DNA quantification. **Methods:** Genomic DNA from 28 volunteers was extracted from 200 ul of EDTA-whole blood using MagNA pure 96 DNA and Viral NA Small Volume Kit on MagNA pure 96 instrument (Roche Diagnostics) according to the manufacturer's specifications. One microliter of extracted DNA was quantified by three distinct methods: UV absorbance (NanoDrop, Thermo scientific), automated microfluidic electrophoresis (TapeStation using genomic DNA screen Tape, Agilent Technologies) and qPCR targeting a 65 bp single copy genomic sequence (absolute quantification of RNase P gene, in-house). Mean DNA concentrations obtained by each method were compared using repeated measures one-way ANOVA with Geisser-Greenhouse correction followed by Tukey multiple comparisons test. Pearson correlations coefficient and Bland-Altman analysis of the tested methods were calculated. **Results:** The DNA yield (mean ± sd) measured by UV absorbance, automated microfluidic electrophoresis and qPCR were 50.71 ± 30.61 ng/ul, 25.24 ± 11.84 ng/ul and 25.37 ± 10.54 ng/ul, respectively. UV absorbance mean was different from automated microfluidic electrophoresis ($p<0.0001$) and qPCR ($p<0.0001$). No difference was observed between automated microfluidic electrophoresis and qPCR ($p=0.96$). Correlations coefficient (r) were: 0.65 ($p=0.0002$) for automated microfluidic electrophoresis and UV absorbance, 0.67 ($p=0.0008$) for UV absorbance and qPCR, and 0.92 ($p=1.35x10-12$) for automated microfluidic electrophoresis and qPCR. Bias (%) ± sd and 95% limits of agreement were 53.98% ± 49.57 and -43.18% to 151.1% between automated microfluidic electrophoresis and UV absorbance, 52.47% ± 51.06 and -47.6% to 152.6% between UV absorbance and qPCR, and -2.01% ± 17.25 and -35.86% to 31.76% between automated microfluidic electrophoresis and qPCR, respectively. **Conclusion:** Here we observed a higher agreement between automated microfluidic electrophoresis and qPCR for genomic DNA quantification. Conversely, both methods showed different results from UV absorbance method. Probably, DNA quantification by UV absorbance is being overestimated due to the co-extraction of RNA. Automated microfluidic electrophoresis and qPCR seems to be comparable (and reliable) quantification methods. However, both methods have their particularities. Automated microfluidic electrophoresis would be adequate for high molecular weight DNA and when information about the DNA integrity is needed (e.g., target NGS sequencing). On the other hand, qPCR would be adequate for fragmented DNA and when information about the presence of PCR inhibitors co-purified during the extraction is necessary (e.g., liquid biopsies and FFPE tissue).

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Evaluation of PD-L1 Expression by Immunohistochemistry with Fluorescent Antibodies from Flow Cytometry

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Background: We describe the use of peroxidase and alkaline phosphatase conjugates of anti-FITC, -PE, -APC and -PerCP antibodies in order to enable the use and reuse of primary antibodies from flow cytometry in immunohistochemistry with cryosections. While multi-parameter flow cytometry is very effective at evaluating the expression of proteins on particular cell types, it does not provide any information about the relative locations of cells in tissues. This can be overcome by fluorescence microscopy, many of the common fluorophores used in flow cytometry are bright but prone to rapid photobleaching, and autofluorescence can also be a problem. Immunohistochemistry with conventional secondary anti-mouse, -rat, etc. antibodies can overcome issues with autofluorescence, but many primary antibodies are from the same species and of the same isotype, which makes it challenging to combine two in a dual color stain. **Methods:** We used anti-fluorescent tag antibodies in order to reuse antibodies from flow cytometry and evaluated the expression of PD-L1 and other markers in tissues by dual color immunohistochemistry. Briefly, cryosections were fixed and endogenous peroxidase was inactivated with sodium azide followed by hydrogen peroxide. Sections were blocked and then stained with the primary antibodies followed by the anti-fluorescent tag antibodies. The substrate for alkaline phosphatase (HistoMark RED) was applied first to generate a magenta signal followed by a substrate for peroxidase (TrueBlue or DAB) to generate a blue or brown/black signal. Afterwards, the molecule proflavine could be used to stain the nuclei yellow. **Results:** On human tonsil sections, it was possible to visualize the expression of PD-L1 in relation to T cell subsets, B cells and other cell types. Weaker expression was simulated by using reduced amounts of primary antibodies such that it was no longer possible to distinguish the staining from autofluorescence by fluorescence microscopy. In those cases, the combination of anti-fluorescent tag antibodies and the TrueBlue substrate was generally still capable of detecting expression. **Conclusion:** Anti-fluorescent tag antibodies enable the use and reuse of antibodies from flow cytometry in immunohistochemistry in order to relatively quickly evaluate where expression is taking place without having to use a separate set of antibodies.

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Analytical Validation of a MALDI-ToF Pharmacogenomic Assay

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2018 AACC Abstract

RELEVENCE: Genotyping of pharmacodynamically relevant SNPs is routinely performed when optimizing patient care for therapeutic drugs and has also been hypothesized to be important to predict susceptibility to drug-misuse disorders. Recent combinations of pharmacogenomic biochemical tools and mass spectrometric methods have allowed for sensitive, economical, and rapid processing of patient samples for pharmacogenomic patient profiling in the clinic. Mass Spectrometry is an analytical technique exceptional for a high level of sensitivity which enables successful analysis of sample sizes up to 2 orders of magnitude smaller than that of traditional RT-PCR. Here, we compare the Agena MassARRAY MALDI-ToF analyzer using the PGx74 SNP panel for genotyping of patient samples previously characterized by RT-PCR. **OBJECTIVE:** The goal of this study was to validate the analytic accuracy and reproducibility of the Agena MassARRAY instrument and PGx74 assay via comparison to previously tested DNA samples with known genotypes. The ultimate goal is to develop a prospective tool to guide opioid usage in patients seeking care from Pain Management providers. **MATERIALS AND METHODS**: We assessed the ability of the Agena MassARRAY 4.0 MALDI-ToF MS instrument to detect SNPs known to be associated with opioid metabolism and response. The Agena PGx74 panel detects wild type and variant SNP sequences as well as copy number variants in 20 genes, including *ABCB1*, *APOE*, *COMT*, *CYP1A2*, *CYP2B6*, *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *DRD2*, *F2*, *F5*, *GLP1R*, *MTHFR*, *OPRM1*, *PNPLA5*, *SLCO1B1*, *SULT4A1*, and *VKORC1*. Archived DNA samples which were previously tested for pharmacogenomic-related SNPs using TaqMan assays were used to assess performance of the Agena PGx74 assay. After multiplex PCR of target regions, a single base extension reaction was performed that allows for simultaneous SNP and copy number variant detection. Accuracy of the PGx74 assay was determined as percent concordance with previous genotypes. **RESULTS**: Ten samples with known *CYP2C19* genotypes were analyzed using the MassARRAY PGx74 multiplex panel. Three specimens were analyzed four times. Two of the three generated *CYP2C19* results that matched the known genotype on all four replicates. The third specimen generated "no call" results on two of the four replicates. The remaining seven specimens were analyzed twice. Six of these generated concordant *CYP2C19* results on both runs while the remaining specimen generated "no call" results on both runs. **CONCLUSIONS:** The Agena MassARRAY demonstrated accuracy and reproducibility in high quality, previously characterized DNA samples. In lower quality samples, the MassARRAY generated "no call" results rather than incorrect results. The combination of highly sensitive mass spectrometric methods and high-throughput pharmacogenomic biochemical tools may allow for the estimation of patient risk for adverse outcomes following use of opioid therapeutics. The ability to rapidly genotype these specimens is of great utility to providers and patients seeking safe and effective pain relief.

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Development of a Type I Diabetes Genetic Risk Array

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Background: Differentiating between Type 1 diabetes (T1D) and Type 2 diabetes (T2D) is challenging due to the increasing incidence of childhood obesity blurring the traditional T1D versus T2D timelines. More young people are getting T2D and T1D can occur at any stage in life, but treatment and patient care pathways are very different depending on diabetes type. Besides this, there has been an increasing number of cases of T1D occurring at old age. Currently available diagnostic tests have several limitations in accurately diagnosing diabetes subtypes. Up to 15% of young adults are wrongly classified and treated. The aim of this study was to consider genetic predisposition as an aid to improve diabetes classification. Genetic predisposition to diabetes is largely determined by the presence of human leukocyte antigen (HLA) genes. A number of Single Nucleotide Polymorphisms (SNPs) that tag these genes have been shown to identify increased risk. Genome-wide association studies have identified additional non-HLA SNPs, robustly linked with T1D. Combining these, a 10 SNP genetic risk score (GRS) was developed which can aid discrimination between T1D and T2D, particularly when used in conjunction with clinical features and autoimmune markers. **Methods:** The assay employs multiplex Polymerase Chain Reaction (PCR) coupled to Biochip Array Technology (BAT, Randox Laboratories Ltd, Crumlin, UK) to genotype 10 SNPs associated with T1D (Oram *et al*.,2016). Multiplex SNP-specific PCR amplicons are hybridised and spatially separated onto a grid of discrete test regions on a biochip, followed by chemiluminescent detection on the Evidence Investigator analyser. Assay run time is <3 hours from DNA template to generation of SNP genotypes. Assay optimisation and specificity was realised using pre-characterised DNA samples and initially validated by testing DNA samples (n=259) provided by University of Exeter, with results compared against genotypes using an alternative method. **Results:** The Type 1 Diabetes GRS array is capable of rapidly detecting all 10 SNPs associated with T1D. Using the biochip array, all 259 samples (2590 genotypes) were in agreement (99.9%) with genotypes predicted by University of Exeter. Samples tested covered all the genotypes linked to 10 SNPs. **Conclusion:** The Type 1 Diabetes GRS array provides a rapid and reliable genotyping test for detecting 10 SNPs associated with T1D. Through an associated algorithm, the array can generate a T1D Genetic Risk Score, which in conjunction with conventional methods, can distinguish T1D from other subtypes. This assay has potential to prevent misdiagnosis of diabetes and facilitate improved diabetic patient management.

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Alterations in hepatic oxylipins, oxidized metabolites of n3 and n6 PUFAs, in liver injury caused by alcohol consumption

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Background: Alcoholic liver disease (ALD), a significant health problem, progresses through the course of several pathologies including steatosis, steatohepatitis, fibrosis, and cirrhosis. There are no effective FDA-approved medications to prevent or treat any stages of ALD, and the mechanisms involved in ALD pathogenesis are not well understood. Bioactive lipid metabolites play a crucial role in numerous pathological conditions, including liver diseases, as well as in the induction and resolution of inflammation. **Aim/Objective**: Herein, a hepatic lipidomic analysis was performed on a mouse model of ALD with the objective of identifying novel metabolic pathways and lipid mediators associated with alcoholic steatohepatitis, which might be potential novel biomarkers and therapeutic targets for the disease. **Methods:** C57BL6/J male mice were fed *ad libitum* control or ethanol (EtOH) liquid diets that contained a high percentage of unsaturated fat (USF, corn-oil enriched) or saturated fat (SF, medium chain triglyceride oil-enriched) for 10 days, followed by a single EtOH administration by oral gavage. Liver steatosis, inflammation, and injury were evaluated. Hepatic lipidomic was performed by HPLC analysis. Mass spectra for each detected lipid mediator were recorded to verify the identity of the detected peak in addition to MRM transition and retention time match with the standard. The data were collected and the MRM transition chromatograms were quantitated. The internal standard signals in each chromatogram were used for normalization for recovery as well as for relative quantitation of each analyte. Statistical significance was determined using Two-Way Analysis of Variance (ANOVA). A p-value of < 0.05 was considered statistically significant. **Results:** We found that EtOH and dietary USF, but not SF, resulted in more severe liver damage, including elevated plasma ALT levels (a marker of liver injury), enhanced hepatic steatosis, oxidative stress, and inflammation. qRT-PCR analysis for macrophage type M1 and M2 cytokine gene expression revealed that M1-associated proinflammatory cytokines (Tnf-α and Il-1beta) were elevated in mice fed the USF+EtOH diet but showed no changes in M2-associated (Tgf-beta and Arg-1) cytokine gene expression. We then performed a targeted lipidomic analysis to measure hepatic levels of bioactive lipid metabolites generated from ω3 and ω6 PUFAs, predominantly via three major enzymatic pathways, LOX, COX and CYP/sEH. A total of 17 oxylipins out of 79 detected metabolites were found to be significantly different (increased) in the USF+EtOH compared to SF+EtOH group, and 21 lipid metabolites were different (19 increased and 2 decreased) between the USF+EtOH and control USF-diet fed animals. Liver injury induced by USF+EtOH was associated with increased levels of oxylipins generally involved in pro-inflammatory responses, including 13-hydroxy-octadecadienoic acid, 9,10- and 12,13-dihydroxy-octadecenoic acids, 5-, 8-, 9-, 11-, 15-hydroxy-eicosatetraenoic acids, and 8,9- and 11,12-dihydroxy-eicosatrienoic acids, in parallel with an increase in pro-resolving mediators, such as lipoxin A4, 18-hydroxy-eicosapentaenoic acid, and 10S,17S-dihydroxy-docosahexaenoic acid. **Conclusion:** The identification of pro- and anti-inflammatory and pro-resolving eicosanoids/oxylipins associated with EtOH-induced liver damage may shed new light into the molecular mechanisms underlying ALD development/progression and might be novel biomarkers and potential new therapeutic targets for ALD. Therefore, further mechanistic studies are warranted.

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Comparison of the *Realtime* HPV HR-S Detection with the Cobas 4800 HPV test for the detection of high-risk types of human papillomavirus

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Background: The *Realtime* HPV HR-S Detection (SEJONG BIOMED, Paju, Korea) is one of the recently developed assays, which is a real-time PCR based test designed for detecting 14 types of high-risk (HR) HPVs (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). This study was to compare the performance of *Realtim*e HPV HR-S Detection to Cobas 4800 HPV test (Roche Molecular Systems, Branchburg, NJ, USA) for the detection of high risk (HR) HPV. **Methods:** A total of 334 cervical swab specimens were retrospectively collected from patients whose mean age was 43.3 ± 12.0 years (22-90 years) between June and September 2016. We tested all the specimens by *Realtime* HPV HR-S Detection and Cobas 4800 HPV test. HPV DNA sequencing was subsequently analyzed to confirm the discordant results. HPV distribution by age, results of Pap test and cervical biopsy for 303 patients was also analyzed and the sensitivity and specificity for high risk lesion and squamous cell carcinoma (SCC) were analyzed. **Results:** Cobas 4800 HPV test one of 13 HR HPV types in 62.0% of specimens, while Realtime HPV HR-S Detection detected in 62.6% of specimens. The overall agreement rate between the assays was 95.2% with 0.937 kappa coefficient. One of the discordant samples was revealed that the result from Cobas was equal to sequencing and the rest 15 samples were revealed that the results from Realtime HPV were equal to sequencing. Sensitivity and specificity of 16, 18 and other high HPV detections were high enough (Cobas 4800: 91.7-100.0% and 98.0-100.0%, and Realtime HPV HR-S Detection: 98.6-100.0% and 100.0%). Sensitivity and specificity for high grade lesion and SCC were similar between at the two tests; 76.9% and 41.9% by Realtime HPV HR-S Detection vs. 80.8% and 43.0% by Cobas 4800 HPV test.For the clinical performance, we calculated sensitivity and specificity of each two tests for pathologic finding of high grade lesion and SCC. *Realtime* HPV HR-S Detection showed 76.9% (56.4-91%) of sensitivity and 41.9% (36.0-47.9%) of specificity while Cobas 4800 HPV test showed 80.8% (60.6-93.4%) and 43% (37.1-49.0%) of those. **Conclusion:** HPV test is getting important at the risk evaluation of cervical cancer. With a methodological development of HPV DNA detection, HPV HR typing is required as well as an accurate HPV detection. In addition, the test has to be validated in a clinical aspect with sensitivity and specificity for the clinical use. *Realtim*e HPV HR-S Detection showed a high agreement rate with Cobas 4800 HPV test and a similar analytical effectiveness with high sensitivity and specificity. Considering the results, *Realtim*e HPV HR-S Detection could be a reliable laboratory testing method for the screening of HPV infections

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An evaluation of Clostridium Difficile Polymerase Chain Reaction on the Roche Cobas 4800.

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Introduction

We compared our current method of toxigenic Clostridium Difficile (*C diff)* testing against the Roche Cobas 4800 system and CE marked Roche C diff test. Our current methodology, validated for in-house use is the TIB MOLBIOL (Berlin, Germany) LightMix *C. difficile* Test, optimised for the Roche LightCycler 2.0 (Basel, Switzerland). Sample preparation of the stool is performed on the Qiagen EZ1 (Venlo, Netherlands). In contrast, the Cobas 4800 system comprises the extraction and purification x480 unit while amplification, detection and result rendition takes place on the z480. The only human intervention clrequired is to transfer the prepared PCR plate from the x480 to the z480 unit.

Materials and methods

We tested 83 anonymised stool samples either retrospectively or as split samples on the Cobas 4800, in accordance with the manufacturer's instructions. The study also included samples spiked with ATCC strains for Ribotype 027, 405 and AI-56. A lower limit of detection challenge was performed using a serially diluted patient sample, ATCC spiked negative stool and ATCC Ribotype 027 spiked negative stool. A challenge for cross-reactivity was performed by spiking samples with up to 1 McFarland equivalent concentrations of various organisms. Assay precision was evaluated using the cross-threshold values of the negative control, internal control values and the positive control values.

Result

The analytical sensitivity and specificity for the Roche Cobas *C diff* test was 96.4% and 96.1% respectively. The lower limit of detection was determined to be less than 100 CFU/mL which is within the manufacturer's claim. Inter-assay precision yielded a coefficient of variation of 1.34% and 1.53% for the internal and positive controls respectively. No false positives were detected with negative samples spiked with *E. coli, B fragilis* and *P mirabilis*.

Conclusion

The Roche Cobas 4800 *C diff* test is able to provide equivalent diagnostic performance on a platform that requires minimal human intervention. Compared to the current method which encompasses several touch-points which increases risk for contamination and mistakes to occur, the 4800 system allows the operator to focus on more complex activities while maintaining high service levels and customer expectations.

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Unbalanced inherited complex chromosome rearrangement in a child with congenital malformations and developmental delay

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Background: Complex chromosomal rearrangements are rare structural aberrations that involve three or more chromosomes and greater than two breakpoints. They are defined as balanced if the chromosomes have the normal chromosomal complement, or unbalanced if there is additional or missing material. Some carriers of complex chromosomal rearrangements appear to have apparently balanced rearrangements but presents abnormal phenotypes. Abnormal phenotypes are associated to cryptic deletions or duplications, or disruption of genes at the site of recombination, which cannot detected by karyotype analysis. With the introduction of microarray analysis as the diagnostic test, large numbers of submicroscopic, pathogenic copy number variants (CNVs) have been uncovered. **Objective:** To report a case of unbalanced complex chromosomal rearrangement paternally inherited detected in a child with multiple malformations. **Case report:** We report 1-year-old boy with psychomotor development cardiopathy, seizures and syndromic facies. He was born to nonconsanguineous and asymptomatic parents, with no history of obortions. Cytogenetic analysis with G-banding was performed on peripheral blood from the child. An apparently balanced complex rearrangement was found: 46,XY,ins(3;7)(p25;q11.23q21.2)ins(1;3)(q32;p24.1p25). Parental chromosomes analysis were performed. The mother's karyotype was normal. The father was found to have a similar apparently balanced complex karyotype: 46,XY,ins(3;7)(p25;q21.11q21.3)?ins(1;3)(q32;p24.2p25). The proband's array-based comparative genomic hybridization (array-CGH) analysis revealed two pathogenic alterations: a 17.4 Mb duplication in 3p26.3-p24.3 [arr[hg19] 3p26.3p24.3(611,636-18,039,381)x3 and a 16.6 Mb deletion in 7q21.11q21.3 [arr[hg19] 7q21.11q21.3 (78,899,856-95,550,252)x1]. Parental array-CGH analysis were normal. The proband's analysis is the first case in which occurred simultaneously a 3p26.3-p24.3 duplication and a 7q21.11-q21.3 deletion, therefore, we cannot associate its findings to any case already described. However, many features described in the literature for the alterations separately are coincident and correlated with the clinic of the evaluated patient. The complex rearrangements identified by karyotype analysis were interchromosomal insertions involving chromosomes 1, 3 and 7. A balanced, interchromosomal insertion is characterized by one chromosome with an interstitial deletion and another chromosome with na interstitial insertion. As there is no reciprocal segment involved, the imbalances that result from segregation can be a segmental monosomy (loss) or trisomy (gain). There is no question that the array-CGH in the clinic has revolutionized the way segmental aneusomies are detected. Developments in array platforms and bioinformatic tools have improved our ability to detect CNVs even in apparently balanced structural rearrangements. However, in spite of these amazing new technologies, a major roadblock remains unchanged. When a copy number gain is detected, it is impossible to determine where in the genome the additional material resides using the array data alone. This case highlights the importance of an integrated approach to cytogenetic analysis and array-CGH analysis.

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HLA-tagging SNP may not be suitable for Celiac Disease risk prediction in Brazilian population

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Background: Celiac disease (CD) is a gluten-sensitive autoimmune disorder, estimated to affect 1:100 people around the world. In Brazil, according to the National Federation of Celiac Associations of Brazil (FENACELBRA), the historical miscegenation may be responsible for a similar proportion of CD cases, although there are no epidemiological studies available to confirm this estimative. CD is the result of the combination of genetic (human leukocyte antigen: HLA) and environmental (gluten) factors. HLA class II genes are strongly associated with CD predisposition, especially the DQ2.5 (DQA1*05/DQB1*02), DQ8 (DQA1*03/DQB1*0302) and DQ2.2 (DQA1*02/DQB1*02) haplotypes. Almost 95% of CD patients carry at least one of the two risk molecules (DQA1 or DQB1). However, up to 40% of healthy people also carries one of these risk factors and their presence alone is not diagnostic of CD. Testing for HLA risk molecules is routinely performed by different methods, e.g. Tagging SNP, PCR-SSOP and MLPA. In our lab, an in-house assay and Tagging SNP are commonly used. The usage of six HLA-tagging SNPs (Monsuur *et al,* 2008) has proven high specificity and sensitivity (>95%) in European celiac populations, but there are no studies using Brazilian population or comparing these methods. **Objective:** This report compares Tagging SNP and our in-house assay in order to assess the agreement between both methodologies as predictive tools for CD risk. **Methodology:** Genomic DNA was extracted from 13 blood or buccal swab samples using QIAGEN kits. The HLA-tagging SNP was performed as Monsuur *et al.* (2008). The in-house assay consists of DQ2 (DQA1*0501 and DQB1*0201) and DQ8 (DQB1*0302) detection, through allele-specific PCR and fragment analysis followed by AmbiSolv® (Thermo Fisher), respectively. **Results and Discussion:** We obtained seven discordant results (54%) between both methodologies. Two patients presented high risk for CD when submitted to our in-house assay but low risk by Tagging SNP. One of these patients was also analyzed by PCR-SSOP and presented high risk for CD. Five patients presented low risk for CD when submitted to the in-house assay but high risk by Tagging SNP. **Conclusions:** The demand for CD genetic tests has increased in the last years, and reliable determination CD genetic predisposition can avoid unnecessary biopsy and gluten-free diet prescriptions. HLA-tagging SNP is a cost-effective population screening method for CD considered as highly accurate, but it has been only validated in Finnish, Hungarian and Italian populations. Our results are a strong indicative that these six HLA-tagging-SNPs may not be suitable for Brazilian population analysis. More patients will be analyzed and also tested by PCR-SSOP to confirm the CD risk correlations between methodologies.

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Marfan syndrome screening using NGS and MLPA in Brazilian patients

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Background: Marfan syndrome (MFS; MIM# 154700) is a rare, autosomal-dominant connective tissue disorder that is caused in the majority of cases by mutations in fibrillin 1 (*FBN*1; MIM# 134797). This gene comprises 66 exons located in the long arm of chromosome 15 at 15q15-q21.1. Mutations in the transforming growth factor-β receptor 1 and 2 genes (*TGFBR*1 and *TGFBR*2) on chromosome 3 have also been identified in a subset of MFS patients who did not carry *FBN*1 mutations. The disorder is characterized by variable manifestations in the cardiovascular, ocular and skeletal systems. Diagnosis is based on familial history of MFS, *FBN*1 mutation analysis and specific phenotypic characteristics, including ectopia lentis, aortic dilatation or dissection and skeletal features. The incidence is currently estimated to be of 1-3 per 10000 individuals, although this could be under-estimated due to misdiagnosis. Next Generation Sequencing (NGS) and Multiplex Ligation-Dependent Probe Amplification (MLPA) have been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance, such as SNPs and large gene duplications and deletions. **Objective:** Investigate the presence of gene mutations in eight Brazilian patients with familial history and clinic diagnostic of MFS. **Methodology:** Patients were tested using the commercial MLPA kits P065-B1 and P066-B2 (MRC-Holland) for *FBN*1, *TGFbR*1 and *TGFbR*2, following manufacturer's instructions and the *FBN*1 gene was sequenced on Ion Torrent PGM sequencer. Variant analysis was performed using Ion Reporter software and detected variants were classified according to 2015 ACMG-CAP guideline. Furthermore, linkage analyses were performed with genetic markers near *FBN*1 allele related to the altered phenotype. **Results and Discussion:** One patient presented a large disruptive heterozygous deletion in *FBN*1 gene from exons 50 to 66. No alterations were detected by MLPA for *TGFBR*1 and *TGFBR*2. A total of 14 (13 SNPs and one deletion) variants were detected considering all patients with four novel variants not currently annotated in public databases such as ClinVar and dbSNP. Three patients presented novel likely pathogenic variants in exons 14, 29 and 64. One patient presented a novel pathogenic variant in exon 16. Eight variants were benign or likely benign and the remaining 2 variants were of unknown significance. No alterations were found for three patients; therefore, the genetic factor contributing for the disease is probably located in their intronic or regulatory regions or in genes other than those analyzed. **Conclusions:** MFS is a genetic disorder with considerable morbidity and mortality, which early diagnosis is essential for the prevention of aortic events. The description of novel variants related to MFS is an important support for medical counseling and diagnosis.

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Analyses on the genotype and phenotype of pediatric deaf patients with cochlear implantation

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Background: National surveys show that the number of handicapped people with hearing impairment in China has reached to more than 27 million. Cochlear device implantation (CDI) is still the only reliable choice for hearing treatment in severe sensorineural hearing loss. This study sought to screen the frequency of mutations and the main types of the mutations, to provide theoretical basis for further analysis on the differences in hearing and speech rehabilitation after CDI in children with different genetic mutations and to provide clinical guidance for children with deafness who need CDI. **Methods:** A total of 42 patients that did not pass newborn hearing screening and were diagnosed as congenital infant deaf had peripheral blood samples collected at the First Affiliated Hospital of Anhui Medical University. Genetic testing was performed by fluorescence PCR on three high-frequency deafness susceptibility hot spot gene mutations: GJB2, SLC26A4 (PDS) and mitochondrial mtDNA 12s rRNA. **Results:** Four out of 42 patients (9.52%) had GJB2 235delC heterozygous mutation, 8 (19.05%) had SLC26A4 mutation (1 case of IVS7-2 A> G homozygous mutation, 1 case of 1174A> T homozygous mutation, 1 case 2168 A> G homozygous mutation with IVS7-2 A> G heterozygous mutation, 3 cases of IVS7-2 A> G heterozygous mutation, and 2 cases of 1174 A> T heterozygous mutation) and 2 (4.76%) had mtDNA 12s rRNA 1555A> G heterozygous mutation. **Conclusion:** Among the 42 cases of pediatric patients with congenital deafness and went through cochlear implantation, the detection rate of the three common deafness gene mutations in China was 33.33%, of which the mutation of SLC26A4 had the highest frequency, GJB2 gene was the next, and the mutation of mitochondrial gene was not common. This study provided a theoretical basis for further analysis of children with different gene mutations in hearing and speech rehabilitation after cochlear implantation, and provides clinical guidance for children with deafness who need cochlear implantation.

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limit of detection of the molecular assays mix® modular giardia for the study of giardia lamblia in coprological samples

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Blackground: The diagnosis of persistent diarrhea due to *Giardia lamblia* can be complicated by the low parasitic burden. The most used diagnostic technique by laboratories, is microscopy, due to its low cost and simplicity. However, traditional microscopy requires time and experience,In recent years, real-time PCR has become an important alternative for the diagnosis of intestinal parasitosis, especially due to its greater specificity and sensitivity to microscopy, an aspect that has been evaluated in patients with acute diarrhea.The aim is to evaluate the detection limit of the LightMix® Modular Giardia Roche Diagnostics molecular technique for the detection of *Giardia lamblia* cysts.**Methods:** We studied 3 samples of stools parasitized with *Giardia Lamblia* and considered microscopically as high parasitic concentration (~840cysts/µL, 1), medium (~490cysts/µL, 2) and very low (~30cysts/µL,3). Serial dilution was performed in duplicate in each sample, microscopically quantifying each one (number of *Giardia lamblia* cysts per microliter) in Burker's chamber. The limit of detection was defined as the lowest dilution that showed a positive result in 100% of the cases.The extraction phase was carried out in MagnaPure equipment (Roche Diagnostics) and for this the samples were diluted 1/10 in STAR Buffer and subsequently pre-treated with MagNAPure Bacteria Lysis-Buffer.Molecular amplification was performed by real-time PCR (Roche Diagnostics) amplifying a 62 bp fragment of the 18s rRNA gene from *Giardia* on a Cobas z480 device.**Results:**

Dilution	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
	C-->Dc	C-->Dc	C-->Dc	C-->Dc	C-->Dc	C-->Dc	C-->Dc	C-->Dc	C-->Dc
Sample, 1	843-->31.63	428-->33.83	227-->34.32	109-->35.30	52-->35.67	23-->36.55	11-->37.21	5-->-	3-->-
848-->31.61	431-->33.85	236-->34.34	113-->35.32	55-->35.71	26-->36.68	12-->37.23	5-->-	1-->-	
Sample, 2	490 -->32.60	241-->34.10	123-->34.98	61-->35.23	30-->36.13	13-->37.17	5-->-	2-->-	
493-->32.61	244-->34.07	125-->35.07	63-->35.25	32-->36.23	12-->37.21	6-->-	4-->-	1-->-	
Sample, 3	31-->36.14	13-->37.19	8-->-	5-->-					
33-->36.25	14-->37.06	7-->-	4-->-	1-->-					

C: Concentration (cysts/µL)Dc: Detection cycleThe median was 12 (11.7-13.3).**Conclusion:** The LightMix® Modular Giardia has a detection limit between 12-13cysts/µL. Therefore, samples parasitized with a low number of cysts of *Giardia lamblia* could be negative in the molecular study of the stools samples of these patients. In addition, the small volume of sample used in the extraction phase, together with the difficulty of breaking the cysts in the pretreatment, may limit the usefulness of molecular techniques as an alternative to conventional microscopy.

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Proficiency testing in NSCLC-related multigene molecular detection by next generation sequencing in China

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Background: Non-small cell lung cancer (NSCLC) is heterogeneous group of carcinomas and stands 70%-80% of cases in lung cancer. As targeted therapies based multiple-gene testing have improved the outcomes of NSCLC patients and decreased cost of next-generation sequencing (NGS) technology have been widespread used as clinical diagnostic assays in numerous laboratories, the accuracy of oncogenic driver mutation detection becomes an indispensable component in genome-directed cancer therapy. Hence, National Center for Clinical Laboratories (NCCL) organized a proficiency testing (PT) for NGS-based multiple genetic detection in NSCLC in 2017,

on the purpose of evaluating the multiple gene testing capability in China. **Methods:** Mimicking NSCLC-related clinical samples were provided to 101 participating laboratories, each with a mock clinical case report. Laboratories were required to use their routine NGS methods to perform the genetic testing and submitted all the variants involved in their detection range within three weeks. Results were evaluated based on predefined marking criteria, the qualified score was set at higher than 90 points. **Results:** Overall, ninety-five laboratories reported their results on schedule. Fifty-two laboratories were considered as qualified while forty-three had completely correct results. More than three hundred errors were divided into 4 parts: 32 false-negatives, 224 false-positives,48 mutation errors and 54 nomenclature errors. The detection rates of SNVs and gene fusion were more than 85% while laboratories had a poor ability in detecting indels, the detection rate of *ERBB2* c.2326delGinsTTAT (p. Gly776delinsLeuCys) was only 62%. **Conclusion:** Our PT results indicated that the detection capability of Chinese laboratories must be improved to further increase the accuracy of NGS-based multigene molecular analysis to ensure reliable results for selection of precision therapy.

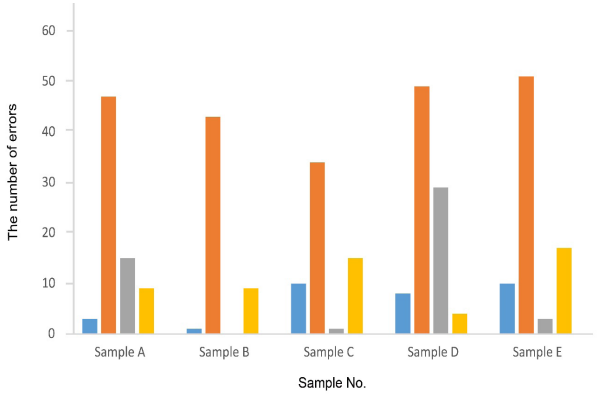

Figure 1: The distribution of errors for each sample in the PT scheme. Four kinds of errors include false-negatives(blue), false-positives(orange), mutation errors(grey)and nomenclature errors(yellow).

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Quantitative detection of *JAK2*-V617F mutation using droplet digital PCR for molecular diagnostics of myeloproliferative neoplasms

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Background: Myeloproliferative neoplasms (MPNs) are chronic neoplastic disorders defined as abnormal increases in mature peripheral blood cells, resulting in the aberrant clonal proliferation of hematopoietic progenitors. MPNs consist of chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytosis (ET), and myelofibrosis (MF). Karyotyping and BCR-ABL rearrangements tests are indicated for diagnostic and prognostic of these disorders. Under negative results, investigation of V617F mutation in the *JAK2* gene is required. JAK2 is a tyrosine kinase that act in signal transduction in hematopoietic cells. The V617F mutation results in the production of a constitutively activated JAK2 protein, which seems to improve the cell survival and increase production of blood cells. This mutation is found in approximately 96% of patients with PV, 50% of individuals with ET and primary MF. Besides the qualitative detection of a V617F mutation, quantification of mutated allele burden provides useful information for classifying subgroups of MPNs and to predict disease-associated outcomes. **Objective:** The aim of this study it was to validate the quantitative detection of the V617F mutation through a high-sensitivity methodology (droplet digital PCR-ddPCR) improving the molecular diagnostics of myeloproliferative neoplasms. **Methods:** Wild-type (≅4000 copies/reaction) and mutated alleles (≅5000 to 0 copies/reaction) of synthetic DNA were combined to stablish the limit of quantification (LOQ) of the test. Blood samples were obtained from health donors (n=2) to determine the false-positive rate and limit of detection (LOD). Twenty-six MPNs samples were selected based on previous results of qualitative V617F test (qPCR) to demonstrate the clinical performance of the ddPCR. DNA samples were collected using QIAamp DNA Mini Blood or Puregene Blood Kits (QIAGEN) and yield was measured by Nanodrop (ThermoFisher). DNA samples (10ng/uL) were submitted to ddPCR using V617F JAK2 Digital PCR Mutation Detection Assay (ThermoFisher) and runned at QX100 Droplet Digital PCR System (BioRad). **Results:** ddPCR detected the presence of the V617F mutation in all dilutions down to 0.06% of mutant alleles. However, LOQ of V617F was achieved at 0.1%, which approximates to 3.4 copies/reaction of mutant alleles. The false-positive rate it was 0.05% and LOD stablished was ≥3 positive droplets to call a V617F positive result (95% CI). To further assess specificity of ddPCR, JAK2 V617F allele burden was measured in 26 DNA samples previously found positive (n=13) and negative (n=13) by qPCR. All positive samples could be classified as positive by ddPCR as well showing different frequencies of allele burden (1.4% to 93.1% of mutant alleles). Among negative samples, 1/13 (7%) patient was positive by ddPCR (0.26% of mutant alleles). This patient had a previously negative BCR-ABL test. **Conclusions:** Our data showed that ddPCR cutoff value was 0.1% of mutant allele in a background of wild-type alleles for an accurate quantitation of JAK2 V617F. In addition, this methodology showed high sensitivity than qPCR for at least 7% of cases. ddPCR was able to identify low levels of V617F mutation from one patient who was negative for qPCR. Therefore, quantitative detection of V617F can be used for prognosis and minimal residual disease monitoring in MPNs patients.

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Regulatory role and clinical significance of circular RNAs in Spinocerebellar ataxia type 3

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Backgrounds: Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) is the most common autosomal dominant spinocerebellar ataxia and one of many inherited polyglutamine (polyQ) neurodegenerative diseases. Nevertheless, the exact mechanism of the disease still remains ambiguous. At present, circular RNAs (circRNAs) have been attracting extensive research interest in different human diseases, which emerged as new key regulators via different biological functions in genetic and epigenetic processes, but it remains largely unknown if they are correlated with SCA3/MJD pathogenesis. Therefore, the objective of this work was to investigate the significance and potential role of circRNAs in SCA3/MJD. **Methods:** Here, we adopted next-generation sequencing(NGS)to examine the expression profile of circRNAs and mRNAs in cerebrospinal fluid(CSF)samples and peripheral blood samples from 11 SCA3/MJD patients and 10 healthy controls. Next, quantitative real-time reverse transcription polymerase chain reaction(qRT-PCR) was performed to validate NGS data. ROC analysis was also used to evaluate the predictive power of candidate circRNAs. In order to elucidate potential functions and signaling pathways involved in the pathogenesis of SCA3/MJD, we applied Gene ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) pathway analysis of the differentially expressed mRNAs and parental genes of circRNAs. Furthermore,after the knockdown or overexpression of candidate circRNA, MTT assay along with flow cytometric assays were used to assess changes in cell viability as well as apoptosis , and Western blot was performed to analyze the disease protein—polyQ-ataxin3 expression in SY-SH5Y/SCA3 cell models. **Results:** Our results showed that circRNAs and mRNAs profiles presented a total of 262 circRNAs and 1001 mRNAs commonly expressed in both CSF samples and peripheral blood samples of SCA/MJD patients. Among them, 14 circRNAs as well as 429 mRNAs were upregulated and 42 circRNAs as well as 549 mRNAs were downregulated in SCA3/MJD group. The expression level changes of 5 differentially expressed circRNAs estimated by qRT-PCR were in accord with NGS data. Moreover, hsa_circ_0019149 (AUC:0.953; 95% CI: 0.911–1.005) were the most upregulated and significantly associated with SCA3/MJD, which could be identified as novel candidate diagnostic biomarker for the disease. Significantly enriched signaling pathways were involved in apoptosis, protein degradation, etc. The viability of cells increased markedly and polyQ-ataxin3 expression decreased following the knockdown of hsa_circ_0019149, whereas overexpression of hsa_circ_0019149 had the opposite effects on cells. **Conclusion:** These findings were the first report of differentially expressed circRNAs in SCA3/MJD, indicating a possible role for circRNAs as potential dynamic monitoring progress biomarkers and possibly original diagnostic or therapeutic targets of the disease. Also, our results provided novel insights into the mechanisms of the pathological process as well as important cues for further functional studies of the disease.

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Ectopia Lentis diagnosed by NGS sequencing of a new pathogenic variant of the FBN1 gene.

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Background: Marfan syndrome (MS) is a systemic connective tissue disease characterized by a variable combination of cardiovascular, musculoskeletal, ophthalmological and pulmonary manifestations. In most cases it is due to mutations in the FBN1 gene (15q21), which codes for an essential connective tissue protein, fibrillin-1. There are identified borderline forms of the disease secondary to mutations in the TGFBR2 gene (3p22), which codes for TGF-beta´s receptor. On the other hand, Isolated *Ectopia Lentis* (IEL) is a rare eye disorder characterized by subluxation of the lens and a significant visual acuity decrease. Its prevalence is unknown, having been described around 90 cases to date, mainly in Europeans. Other manifestations include: congenital anomalies of the iris, spherophakia, abnormal iridocorneal angle, iridodonesis, coloboma of the lens, refractive errors, cataracts of early onset, amblyopia, displacement of the pupils (*ectopia lentis et pupillae*). The disease is due to recessive mutations in the ADAMTSL4 gene (1q21.2) and dominant mutations in the FBN1 gene (15q21.1). By definition, patients with IEL have no associated systemic abnormalities, although cardiac and skeletal examinations should be performed to help exclude Marfan syndrome. **Objective**: This report describes a case of a patient with crystalline subluxation and other ocular anomalies and suspicion of Marfan syndrome, which is finally diagnosed of IEL by NGS sequencing. **Case report:** 5 years female patien, without family history of interest. Size: 116.1cm (p76, 0.71 SD), weight: 16.9kg (p18, -0.92 SD), BMI: 12.54% (p7, -1.54 SD), body surface: 0.74m2, Tanner 1. Presents, in the right eye : superior subluxation of the lens with coloboma of the same, phacodonesis (lens *tremor*), amaurosis (total blindness), endotropia (deviation of the eye) intermittent to + 10º; left eye: superior subluxation of the lens, phacodonesis, decreased visual acuity (0.5 PIGAS-SOU). The electrocardiogram and Doppler-echocardiogram showed no pathology. **Methods**: NGS sequencing of the coding regions of the FBN1, TGFBR1 and TGFBR2 genes was performed: DNA amplified by multiplex PCR with the Ion *AmpliSeq* kit, *Ion Torrent* platform sequencing, bioinformatic analysis using *Thermo Fisher Scientific Variant Reporter*, with an average coverage obtained from 616.987X, covering 96.03% of the coding regions at 20X by massive sequencing and the rest by capillary sequencing. **Results**: A variant of uncertain clinical significance c.6801C> A (p.Asn2267Lys) is detected in heterozygosis in exon 56 of the FBN1 gene (sense change mutation). In order to determine if this change is inherited or *de novo*, genetic study was done to the parents and the brother, who didnt´t present the variant. **Conclusions**: This alteration has not been previously described in the literature, so it´s considered of unknown clinical significance. However, several bioinformatic tools specialized in the prediction of the effect of mutations (*Polyphen, SIFT, MutationTaster*) classify it as deleterious. Compatible *in silico* prediction, the fact the the variant found in our patient is *de novo* and the pathophysiology context of the FBN1 gene alterations and the proteins it encodes, we assume that the mutation c.6801C> A (p.Asn2267Lys) of the FBN1 gene is responsible for the diagnosis of isolated ectopia lentis.

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End-to-end automated workflow for simultaneously genotyping of multiple clinically relevant single nucleotide polymorphisms

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Background: Traditionally, the molecular pathology laboratory relies on manual techniques. Especially, when laboratory-develop tests (LDT) or tests of low demand are considered (e.g., genotyping of clinically relevant nucleotide variations of the human genome). However, end-to-end automated solutions for these tests are now available leading a paradigm shift in the field. Thus, the present study aimed to validate an automated and laboratory information system (LIS) integrated qPCR workflow for simultaneous detection of seven clinically relevant single nucleotide polymorphisms. **Methods:** This automated workflow validation included EDTA-whole blood from volunteers with known results for factor V Leiden (n=239), G20210A mutation in prothrombin gene (n=154), HLAB-27 allele status (n=235), C−13910T mutation in lactase gene (n=73), C677T mutation on methylenetetrahydrofolate reductase gene (MTHFR) (n= 99) and C282Y and H63D mutation in hemochromatosis gene (HFE) (n=52). The complete automation workflow was provided by the flow classic solution (Roche). The platform consisted of two automated liquid hander workstations (ALHW) (one for primary samples aliquoting and the other for qPCR set up), an automated nucleic acid extractor (Magna Pure 96) and a 384-well thermocycler (Light Cycler 480II). During the workflow, the instruments integrator software receives a sample worklist from the LIS (including samples barcode and the test to be performed). The first ALHW identifies the samples introduced by the operator allowing the creation of worklists for the downstream instruments and transfers 50ul of whole blood for an extraction plate. Genomic DNA is purified by the automated nucleic acid extractor. Simultaneously, the PCR reactions are set up by the second ALHW. For that, the instrument is loaded with sybr green qPCR master mix, ARMS qPCR primers (n= 21) and empty tubes for the allele-specific reaction mixture preparation (n=14) (according to with the worklist provided by the first ALHW). After the reaction mixture preparation and distribution into the qPCR plates, the extracted DNA and controls DNA (comprising all possible genotypes) are loaded into the equipment and transferred their specific qPCR wells. After the thermocycling, the amplification data is transferred to the integrator software, inspected by the operator together with the melting curves and the approved results are sent to LIS without any typing. The workflow can process 82 samples total (irrespective of the requested test) in 5 hours, have complete traceability, and for its validation, the obtained by results was compared with the expected results. **Results:** The automated workflow attributed the expected genotypes for all samples in all instances: 223 GG, 13 GA and 3 AA for factor V Leiden; 144 GG, 10 GA and 0 AA for Prothrombin G20210A; 29 positive and 206 negative for HLA-B27; 28 CC, 38 CT and 7 TT for Lactase C−13910T; MTHFR 43 CC, 46 CT, and 10 TT for MTHFR C677T and 48 GG, 3 GA, 1 AA for HFE C282Y and 35 CC, 14 CG, 3 GG HFE H63D mutation in hemochromatosis gene. **Conclusion:** The proposed automated qPCR workflow could accurately genotype seven distinct SNP. Its full automation confers higher safety and quality for the process.

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The serum has a higher yield of Janus kinase 2 V617F somatic mutation compared to the paired EDTA-whole blood sample.

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Background: Janus kinase 2 (JAK2) V617F is a somatic mutation of blood cells, usually detected in genomic DNA extracted from nucleated cells of the whole blood. As coagulation releases genomic DNA of these cells to serum, this specimen could be used as a source of DNA for the JAK2 V617F detection and quantification. This study aimed to test if JAK2 V617F can be detected reliably in serum and investigate if the percentage of the JAK2 mutant allele in serum is comparable to the rate found in whole blood. **Methods:** This study enrolled 88 subjects, 28 with positive for JAK2 V617F and 60 healthy volunteers. Paired EDTA-whole blood and serum samples were collected from each participant in two 4 mL tubes: EDTA-whole blood and Vacuette Z serum sep with clot activator (both from Greiner Bio-One). Genomic DNA was extracted from 500 ul of serum or 200 ul of EDTA-whole blood using Magna Pure 96 instrument (Roche) or EasyMAG (Biomerieux) according to the manufacturer's recommendations. EDTA-whole blood and serum extracted genomic DNA were quantified by a qPCR. The JAK2 wild-type (WT) and mutant (MUT) alleles were assessed by separated AS-qPCR reactions using the StepOne real-time system. The RNase P was co-amplified in both reactions to function as a normalizer gene. The percentage of JAK2 MUT allele was calculated by the delta-delta Cq method using JAK2 WT allele as comparator sample. The agreement between the rate of JAK2 mutation in EDTA-whole blood and serum was calculated using Pearson correlation and Bland-Altman analysis. The mean percentage of JAK2 MUT in paired specimens was compared by paired t-test. **Results:** Qualitatively, there was a complete concordance between serum and EDTA-whole blood results (28 positives and 60 negatives). Quantitatively, there was a correlation between serum and EDTA-whole blood results ($r=0.987$, $p<0.0001$). The JAK2 MUT yield bias between both specimens where -3.78% and the 95%CI agreement was -15.37% and 7.77%. The mean percentage of JAK2 MUT in serum was 33.89% (31.09) in serum and 30.11% (27.57) in the EDTA-whole blood ($p=0.0021$). **Conclusion:** JAK2 V617F mutation could be reliably detected in serum by qPCR using delta-delta Cq method. The JAK2 MUT allele burden in serum was significantly higher than in EDTA-whole blood. Serum results tended to be 3.78% higher than the EDTA-Whole blood results. This finding can be explored to increase the mutant allele detection rate in the routine.

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Simplified workflow for PML-RARA quantification in whole blood by automated nucleic acids extraction, multiplex one-step RT-qPCR and ΔΔCq method

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Background: Molecular testing for the PML-RARA fusion gene by RT-qPCR is a substantial approach for monitoring the response to therapy and predict relapse of patients with acute promyelocytic leukemia. The most frequent fusions subtypes are bcr1, bcr2 and bcr3 (found in ~55, ~5 and ~45% of the patients, respectively). The quantification assays for these transcripts have been standardized at international level. However, they are laborious and often require multiple steps that are not user-friendly. Thus, the objective of this study was to validate a simplified multiplex bcr1, bcr2 or bcr3 fusion quantification in whole blood and bone marrow by RT-qPCR. **Methods**: We used patient's specimens, or known amounts of synthetic fusion RNAs spiked into the negative samples. Red blood cells were removed from buffy coats prior extraction using PharmLyse (BD Biosciences). Nucleic acids were extracted on Magna Pure 96 (Roche). The reaction was designed to quantify bcr1/bcr2 or bcr3 and ABL1 RNA in a multiplexed one-step RT-qPCR reaction performed with QuantiNova RT-qPCR master mix (Qiagen) on LightCyclers 480II or z480 (Roche). Europe Against Cancer primers/probes for bcr1, bcr3, and ABL1 and Chen et al. 2015 for bcr2 were used. Ipsogen's (Qiagen) standard curves were used for the absolute quantification of bcr1/bcr2, bcr3, and ABL1. The applicability of the ΔΔCq method was evaluated analyzing the amplification efficiencies retrieved from a serial dilution of the synthetic RNAs corresponding to the studied molecular targets. The limit of detection (LoD) was determined by using probit regression analysis to a serial dilution of known amounts of each target. Precision was evaluated by CLSI EP12-A2 method on samples spiked with the high, medium and low amount of targets RNA in triplicate for seven days. The accuracy was assessed with the spike-recovery method by using Pearson correlation coefficient (r) and Bland-Altman analysis. **Results**: The median (Max-Min) of ABL1 achieved by the proposed workflow was $9.6x10^4$ ($3.98x10^3$-$7.11x10^5$) copies/sample. bcr1/bcr2 or bcr3 and ABL1 RT-qPCR efficiencies did not differ significantly in all tested occasions (95.18% for bcr1/bcr2; 97.95% for bcr3; 98.6% for ABL1) meaning that the ΔΔCq method is applicable. The LoD were 0.026% (95%CI 0.015-0.078) and 0.014% (95%CI 0.010-0.031) of PML-RARA/ABL1 for bcr1/bcr2 and bcr3, respectively. Imprecision values for low, medium and high levels of fusion synthetic RNAs, expressed as CV(%), were 4.98%, 10.65% and 11.77% for bcr1 and 5.03%, 9.56% and 27.46% for bcr3. In The Spike-recovery experiment, expected and observed measurements revealed a correlation coefficient (r) of 1.0 for bcr1/bcr2 and bcr3, and bias of 0.011 Log for bcr1/bcr2 and 0.035 Log for bcr3. Cross-reactivity between bcr1/bcr2 and bcr3 assays was not observed. **Conclusion**: We described a reliable and user-friendly workflow for PML-RARA bcr1/bcr2 and bcr3 fusion quantification in whole blood and bone marrow that reduces the number of steps proposed by the current guidelines. The proposed workflow showed acceptable sensitivity, precision, and accuracy. The assay reached the sensitivity at least 4.4-Log reduction based on the bcr1 and bcr3 positive samples. The main drawback in this study is the lack of bcr2 patients' samples.

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Rapid Somatic Mutation Testing in Colorectal Cancer Using a Fully Automated System and Single-Use Cartridge: A Comparison with Next-Generation Sequencing

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Background: Molecular tests have been increasingly used in the management of various cancers as more targeted therapies are becoming available as treatment options. The Idylla™ system (Biocartis, Mechelen, Belgium) is a fully integrated, cartridge-based platform that provides automated sample processing (deparaffinization, tissue digestion and DNA extraction) and real-time PCR-based mutation detection with all reagents included in a single-use cartridge. **Objective:** This retrospective study aimed at evaluating the Idylla™ KRAS and NRAS-BRAF-EGFR492 Mutation Assay cartridges against next-generation sequencing (NGS) using colorectal cancer (CRC) tissue samples. **Methods:** Forty-four archived formalin-fixed paraffin-embedded (FFPE) CRC tissue samples previously analyzed by targeted NGS were tested on the Idylla™ system. Among these samples, 17 had a mutation in *KRAS,* 5 in *NRAS,* and 12 in *BRAF* as determined using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific) . The remaining 10 samples were wild-type for *KRAS*, *NRAS* and *BRAF*. Tow 10 µm FFPE tissue sections were used for each Idylla™ run, one for the KRAS cartridge and one for the NRAS-BRAF-EGFR492 cartridge. All cases met the Idylla™ minimum tumor content requirement for *KRAS, NRAS*, and *BRAF* (≥ 10%). Assay reproducibility was evaluated by testing commercial standards derived from human cell lines, which had an allelic frequency of 50% and were run in triplicate. **Results:** The Idylla™ system successfully detected all mutations previously identified by NGS in *KRAS* (G12C, G12D, G12V, G13D, Q61K, Q61R, A146T), *NRAS* (G12V, G13R, Q61H), and *BRAF* (V600E). Compared to NGS, Idylla™ had a sensitivity of 100% (CI 90 – 100%). No mutations in the wild-type samples were detected by Idylla™, except for one sample that showed an *NRAS* mutation upon initial testing. Idylla™ testing was repeated twice on additional sections from this sample and it was negative. The negative result was further confirmed by another NGS method. Analysis of the horizon mutated control samples demonstrated agreement with the expected result for all samples and 100% reproducibility. The Idylla™ system produced results quickly with a turnaround time of approximately 2 hours. For certain mutations, Idylla™ did not distinguish between mutations occurring in the same codon (e.g. p.Q61R/L in *KRAS*, G12A/V in *NRAS*, and V600E/D in *BRAF*). **Conclusion:** The fully automated Idylla™ system offers reliable and sensitive testing of clinically actionable mutations in *KRAS, NRAS* and *BRAF* directly from FFPE tissue sections. Its simplicity and ease of use compared to other available molecular techniques make it suitable for small centers that lack highly trained staff and molecular expertise. Additionally, it can complement NGS and other molecular testing systems at larger diagnostic centers by providing significantly faster turnaround times.

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Absolute Quantification Of Graft-Derived Cell-Free DNA As A Marker Of Rejection And Graft Injury In Kidney Transplantation - Results From A Prospective Observational Trial

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Background: Graft-derived cell-free DNA (GcfDNA), a promising new, noninvasive biomarker of allograft rejection and injury status, was investigated in renal transplant (RTx) patients. **Methods:** In a prospective observational trial, GcfDNA was evaluated at prespecified visits in 88 RTx patients, followed over at least one year post transplantation. Relative percentage (GcfDNA%) and absolute quantification of GcfDNA copies (GcfDNAcp/mL) were performed as previously described [Beck J et al, Clin Chem 2014; 60 (Suppl.): S194-S195]. Biopsies were obtained upon clinical suspicion of acute rejection. GcfDNA results were compared between patients with and without positive biopsies. Data were analyzed using R 3.4.3 (packages: base, nparcomp). Pearson correlations were performed on log transformed data. **Results:** In patients (N=30) without subsequent rejection, active infections, or interventions, GcfDNA was highly elevated (median: 367 cp/mL; 4.20%), presumably due to ischemia/re-perfusion damage, in day 1 post RTx samples (n=10). In all 30 patients GcfDNA values decreased over the first 30 days to a baseline median of 19 cp/mL (0.20%), where it remained throughout the one year observation period. In patients (N=14) with samples (n=21) drawn during biopsy-proven acute rejection (BPAR) periods, median GcfDNAcp/mL was 5-fold and median GcfDNA% 2.6-fold higher (86 cp/mL; 0.68% respectively) than the medians observed in samples (n=267) from 62 clinically stable patients without rejection (17 cp/mL; 0.26%). These comparisons were confirmed by GcfDNA medians in 5 patients with negative biopsies (14 cp/mL; 0.18%). Both GcfDNAcp/mL and GcfDNA% were significantly different between patients with BPAR and apparently stable patients ($p<0.0001$). To compare the diagnostic accuracy of GcfDNAcp/mL and GcfDNA%, the area under the ROC curves (AUC) were calculated in 76 patients. GcfDNAcp/mL (0.88, 95% CI: 0.82-0.92) better discriminated between patients with BPAR and clinically stable patients than did GcfDNA% (0.81, 95% CI: 0.73-0.87). Plasma creatinine was not an independent marker, as it was used clinically as an indication for biopsies. Youden-index (YI)-based diagnostic sensitivity was 90% for GcfDNAcp/ml, and 86% for GcfDNA% obtained from ROC curves. Diagnostic specificity was 76% for GcfDNAcp/mL, and 74% for GcfDNA%. The threshold at maximum YI was 37 for GcfDNAcp/mL, and 0.43 for GcfDNA%. The correlation between GcfDNAcp/mL and GcfDNA% was r=0.75. Creatinine showed a moderate correlation with GcfDNA (cp/mL: r=0.44; %: r=0.41). In a selected patient subgroup (N=25) without clinically suspected rejection and a change of tacrolimus concentrations >60%, in samples (n=78) collected at ≥3 consecutive visits there was a negative correlation (r=-0.49) between tacrolimus and GcfDNAcp/mL. This sug-

gests that GcfDNA may detect silent graft damage due to under-immunosuppression which might increase the risk of de novo DSA formation and subsequent graft loss. **Conclusion:** This is the first systematic comparison of GcfDNAcp/mL with GcfDNA%. Absolute GcfDNA quantification allowed for a better discrimination than GcfDNA% of RTx patients with acute rejection and graft injury, due to less influence of recipient cfDNA variations, and may facilitate personalized immunosuppression.

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Validation of pre-analytical procedures of liquid biopsy samples for investigation of the *EGFR*-T790M mutation by ddPCR in NSCLC Brazilian patients

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Background: Non-small cell lung cancer (NSCLC) represents approximately 85% of all lung cancer types with 5% of survival rate. The T790M mutation in *EGFR* gene accounts for nearly 50% of the acquired resistance to tyrosine-kinase inhibitors (TKIs). Circulating-free tumor DNA (cfDNA) harboring T790M can be detected even at low concentration in plasma. Pre-analytical best practices is mandatory to ensure optimum yield and quality of the cfDNA. **Objective**: The aim of this study it was to validate the pre-analytical steps and establish the analysis protocol of droplet digital PCR (ddPCR) for measuring the levels of T790M mutation in NSCLC liquid biopsy samples. **Methods**: Plasma obtained from 5 health volunteers and 13 commercial controls (Horizon) with different T790M concentrations it were used to determine the limit of detection (LOD) and limit of quantification (LOQ), respectively. Patient samples were collected from different regions of Brazil to demonstrate the clinical performance of the test. *Training Set:* it were collected blood samples in EDTA (4mL) and PAXgene Blood cfDNA (PreAnalytix) tubes (16 mL) from 5 NSCLC patients. *Validation Set:* blood samples from 38 NSCLC additional patients were collected in EDTA tubes (4mL). EDTA samples were centrifuged, plasma was aliquoted and sent frozen to our center. One aliquot (2mL) of each EDTA sample were processed for qPCR cobas® EGFR Mutation Test v2 (Roche). PAXgene tubes blood samples it were send in room temperature and processed 24hs, 72hs or 168hs after collection. cfDNA samples (EDTA and PAXgene) were collected using QIAamp MinElute Virus and QIAamp Circulating Nucleic Acid kits (both QIAGEN) and yield measured using Qubit 1.0 (ThermoFisher). All cfDNA samples were submitted to ddPCR using T790M ddPCR Mutation Assay (BioRad) and QX100 Droplet Digital PCR System (BioRad). **Results:** The false-positive rate it was 0.12% and LOD stablished was ≥5 positive droplets to call a T790M positive result (95% CI). The expected results for control samples were 0.05, 0.5 and 5% of mutant allele frequency and the observed results were 0.053, 0.63 and 5.4% (r^2=0.99) with mean LOQ of 0.57%. Among training set, cfDNA concentration did not show significant statistically difference regardless time-point processing (p=0,6489) and collection tubes (p=0,07). cfDNA yield it was 10 times higher after virus kit collection (mean 0,52 ng/uL) compared to circulating kit (mean 0,05 ng/uL). All these patients were negative for T790M by both PCR methodology, however 4/38 (10%) patients from validation set were positive by qPCR (SQI ≤5) and negative for ddPCR. **Conclusions**: cfDNA collection using virus kit was superior compared to circulating kit. Four patients from validation set (10%) showed discordant results. This could be explained due to the loss of stability of cfDNA from frozen plasma. In addition, recent data has shown inverse correlation between qPCR results with SQI ≤5 and ddPCR T790M negative results. Our data showed that ddPCR is a powerful methodology for detection of low levels of T790M in NSCLC patients, however pre-analytical best practices is crucial for cfDNA stability and ddPCR sensitivity in liquid biopsy samples. COBAS qPCR results with SQI ≤5 should be revised.

Wednesday, August 1, 2018

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

Nutrition/Trace Metals/Vitamins

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Serum Level of Some Micronutrients as a Biomarker of Immunity in Antiretroviral-naïve HIV-infected Individuals

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Background: HIV infection may lead to micronutrient deficiencies. These micronutrient deficiencies may affect the risk of having HIV/AIDS and low $CD4^+$ count level in the patients which is a measure of their level of immunity. This study was to correlate the $CD4^+$ count in antiretroviral-naïve patients with the serum levels of some micronutrients as measures of relationship between immunity and nutrition/malnutrition. **Method:** Ninety consecutive newly diagnosed HIV/AIDS patients (age 18-59 years) attending the clinics were recruited for this descriptive cross sectional study. Ninety apparently healthy, screened blood donors were enrolled for comparison. Blood samples were collected from the control subjects and the patients before HAART treatment and were assayed for serum zinc, selenium, copper, manganese and magnesium using atomic absorption spectrophotometer (AAS). Vitamin B_{12} level was measured using high performance liquid chromatography (HPLC). **Results:** Mean serum vitamin B_{12} was significantly higher in the study participants than the controls (304.59±86.31 pmol/l and 279.77±81.58 pmol/l respectively, p=0.036); while the participants had significantly lower mean serum zinc (14.25±2.93 µmol/l and 14.58±3.69 µmol/l respectively, p= 0.493), significantly lower selenium (0.38±0.08 µmol/l versus 0.78±0.22 µmol/l, p <0.001), manganese (7.06±0.87 µmol/l versus 11.23±3.27 µmol/l, p <0.00), and magnesium (1.02±0.21 mmol/l versus 1.21±0.28 mmol/l, p <0.001) values when compared with the controls. Mean concentration of copper is similar in both participants and controls (18.88±3.1 µmol/l and 18.82±5.12 µmol/l, p= 0.921). There was no correlation between the micronutrients levels and $CD4^+$ count, however, there are strong positive correlations between the serum levels of zinc and copper; zinc, selenium and magnesium; zinc and selenium; copper and magnesium (p values, <0.001 respectively). Multivariate regression analyses among variables with significant correlations showed that all micronutrients were independent predictors of one another (p values of <0.001). Significant positive correlation exists between duration of HIV infection and vitamin B_{12}. There were significant differences in the mean values of vitamin B_{12} among the WHO categories of three degrees of immunosuppression (based on $CD4^+$ count; Anova, p=0.047). The highest concentration was in those with mild immunosuppression, CDC category 1($CD4^+$ ≥500 cells/µl; vitamin B_{12}: 324.1±89.9 pmol/l); declining in those with moderate immunosuppressive state, category 2 ($CD4^+$ 200 – 500 cells/µl; vitamin B_{12}: 317.9±74.5 pmol/l, with multiple comparison (post hoc) analysis p=0.047 between category 1 and 2), and lowest in severe immunosuppression, category 3 ($CD4^+$ < 200 cells/µl; vitamin B_{12}: 274.1±88.2 pmol/l, with multiple comparison (post hoc) analysis p=0.029 between category 2 and 3). The mean difference in vitamin B_{12} between moderate- and severe immunosuppressive state was greater than the mean difference between mild- and moderate immunosuppression. **Conclusion:** HIV/AIDS results in depletion of serum micronutrients with strong positive correlations between their serum levels. Although serum levels of micronutrients may not be qualified as direct markers or surrogates for $CD4^+$ count in antiretroviral-naïve HIV-infected patients, serum vitamin B_{12} could differentiate between mild- to moderate and severe immunosuppressive states; inverse relationship exists between serum levels of vitamin B_{12} and HIV/AIDS disease severity and progression.

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Impact of new values for vitamin D in Brazil assessed in sampling of a reference laboratory

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Background: In Brazil, hypovitaminosis D has been documented in several regions of the country, which justified a critical analysis of diagnostic criteria by a committee of specialists from the Department of Bone Metabolism of the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) for the development of recommendations based on scientific evidence available from current literature on vitamin D. The main change was the adoption of the vitamin D sufficiency criterion to values above 20ng/mL for healthy individuals aged 19 to 60 years versus the previous 30ng/mL. Current criteria consider sufficiency between 30ng/mL to 60ng/mL for risk groups and aged above 60. The study aims to analyze the difference between the adopted criteria, the behavior and the impact in this sampling. **Methods:** Data from 231,830 vitamin D analyses were obtained in adults aged 18-90 years from the southeast region of the country, performed at a reference laboratory, on Abbott-Architect platform, for a period of one year (September 2016 to 2017). Sampling was categorized according to gender, age and ranges of values used for vitamin D in clinical evaluation. The results were subject to a descriptive statistical evaluation and the Mann-Whitney and Kappa tests were applied to estimate the agreement between the previously adopted and current ranges of vitamin D suitability. A significance level of 5% (p <0.05) was adopted in the statistical software SPSS®, version 19.0 and STATA®. **Results**: Sampling is very homogeneous, but non-Gaussian. The mean age of this population was 52,93 years old; the median and the mean ± SD of the vitamin D concentration was 29.65ng/mL and 28.60 ± 10.81 ng/mL, respectively. It was observed that the majority were female (76.91%) and the predominant age group was between 19 and 59 years old (62.36%). The parameters of adequacy for the current levels of vitamin D were evaluated in frequency and percentage, obtaining the following: Insufficient or deficient (<20ng/mL) 10.2% [n=23647]; Sufficient (20 to 29ng/mL) 43.2% [n=100150]; Sufficient and recommended for adults above 60 years (> or = 30ng/mL) 46.6% [n=108033]. The concentration of vitamin D in females vs. male was lower, 28.20 ng/mL vs. 28.40 ng/mL (p = 0.003). Similarly, among individuals aged 19-59 years old the dosage was also lower, 28.10 ng/mL, when compared to the elderly, 28.60 ng/mL (p <0.001). It was observed that there is a low agreement (0.210) between the two classifications, with statistically significant values (p <0.001). **Conclusions:** It is well established that the parameters diverge as for the current and the previously established ranges. We did not analyze clinical criteria or use of replacement medication, which may have an effect, especially in the elderly population data. However, there is a significant difference, in the age group above 60 years old, higher levels as predicted by the current sufficiency criterion. The study provides the impact of this transition of criteria regarding the clinical decision, notably in the southeast region of Brazil, since previously 53.4% of the dosages would be insufficient, contrasting with the current 10.2%.

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Holotranscobalamin still in debate.

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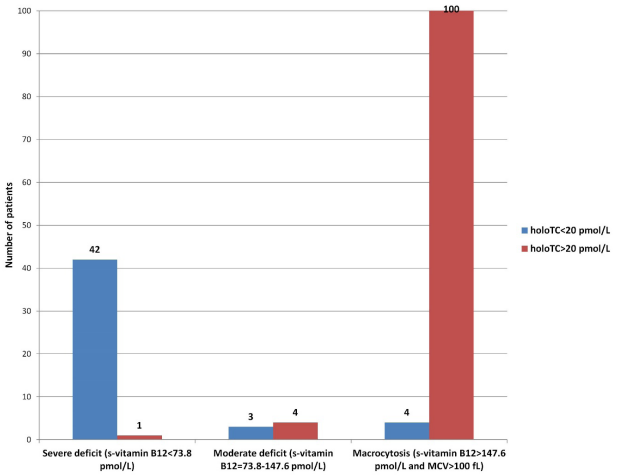
Background: The diagnosis of vitamin B12 deficit is still a challenge for the clinical laboratory since serum vitamin B12 assay (s-vitamin B12) is not standardized. Methylmalonic acid (MMA) and total homocysteine (HCt), the ultimate tests to confirm deficiency, are expensive and not available in daily practice. Holotranscobalamin (HoloTC) might be a more reliable indicator of intracellular vitamin B12 status than s-vitamin B12. The aim was to study holoTC in three groups of primary care patients classified according to s-vitamin B12 and MCV values. **Methods:** The Laboratory located in a Community University Hospital covers a Health Department of 216210 inhabitants. Participants were primary care patients classified into two groups depending on s-vitamin B12<73.8 pmol/L, between 73.8 and 147.6 pmol/L, named "severe" and "moderate vitamin B12 deficit", and a third group with MCV>100 fL and s-vitamin >147.6 pmol/L, named "macrocytosis group". S-vitamin B12 was measured through immunoassay on Modular E170 (Roche Diagnostics, Switzerland), and holoTC by the Abbott Architect Assay (Abbott, USA), with a deficiency cut off of 20 pmol/L. Each patient sample was tested for s-vitamin B12 and holoTC and results were compared.

Results: There were 43 and 7 patients with severe and moderate deficit, respectively; and 104 in the macrocytosis cohort. Among the 43 patients with severe vitamin B12 deficit, 42 had holoTC deficiency, showing 97.7% of coincidence. Only 3 had low holoTC values in the moderate group, with 42.8% coincidence. Among the 104 patients with macrocytosis, only 4 (3.8%) had low holoTC (Figure). **Conclusion:** The high concordance between serum vitamin B12 and holoTC in severe vitamin B12 deficiency and macrocytosis groups shows that any of those markers might be appropriate when dealing with severe deficiency and macrocytosis with non-pathological s-vitamin B12 values. However in moderate deficiency, the use of additional MMA or HCt might be still necessary.



B-298

Method comparison of two different Vitamin D immunoassays by assessing Chinese serum samples

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Background: Total 25-hydroxyvitamin D [25(OH)D] is the most reliable indicator of vitamin D status. Radioimmunoassay kit was used in many large-scale surveys, like China National Nutrition and Health Survey (CNNHS) 2010-2012. On the other hand, enzyme immunoassay kit was widely applied in many researches as well. The application of different analyzing methods brought difficulty for cross comparison of researches. To evaluate the difference between radioimmunoassay and enzyme immunoassay, we compared the most widely used enzyme immunoassay kit and radioimmunoassay kit through assessment of human serum samples from the Chinese population in this study. **Methods:** In total, 653 Chinese serum samples were tested using the IDS 25-Hydroxy Vitamin D EIA kits and DiaSorin 25-Hydroxyvitamin D RIA kits. **Results:** Spearman's rank correlation coefficient (rho) between DiaSorin-RIA and IDS-EIA was 0.7509. The Cusum test showed no significant deviation from linearity for DiaSorin-RIA vs IDS-EIA (P = NS). The Bland-Altman plots were constructed for all evaluated samples to determine the between-assay bias. The 25(OH)D concentrations obtained by IDS-EIA were lower on average (mean bias, -3.2 ng/ml, -6.4%) than the results of DiaSorin-RIA. The weighted kappa values was 0.527 in the assessment of vitamin D deficiency (<20 ng/mL). **Conclusion:** The results indicated acceptable correlation between DiaSorin-RIA and IDS-EIA. They can be recommended for routine use in the Chinese population.

B-299

Levels of Trace Elements and Their Binding Proteins in the Blood of Rheumatoid Arthritis Patients in Saudi Arabia

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Background: Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory joint disorder affecting about 1% of the world population. The severity of this disease may vary from mild arthralgias to destructive erosive disease involving all joint of upper and lower extremities. The pathogenesis of RA is not clearly understood. Several studies have shown altered levels of trace elements in the blood of RA patients as well as their deposition in synovial membranes. **Aim**: This study was aimed to determine the levels of serum iron, copper and zinc; and their binding proteins, hemoglobin, ferritin and albumin of RA patients in a tertiary care referral hospital in Saudi Arabia. **Method**: The RA patients were graded into 3 categories (mild, moderate and severe) using the standard procedures. The study included 34 patients and 17 matched controls. Clinical data and medical history were recorded for all subjects. Blood samples were collected from all subjects and serum concentration of trace metals, zinc, copper were analyzed by Atomic Absorption Spectrophotometry. Iron, ferritin, and albumin were analyzed on Roche Cobas system. Hemoglobin was measured on Sysmex XN system. **Results**: Our results showed a significant decrease (P<0.01) in serum iron (5.11±1.25 vs 11.35±1.25 umol/l) and zinc (10.62±0.95 vs 13.50+0.56 umol/l) levels in RA patients of severe category versus normal subjects. On the other hand serum copper was found to be significantly higher in RA patients (21.59±0.88 umol/l) as compared to controls (17.13±0.87 umol/l). Among the proteins, the level of serum ferritin was significantly higher (P<0.001) whereas the levels of hemoglobin and albumin were significantly lower (P<0.05 and P<0.01 respectively) in RA patients. The regression analysis about the levels of trace elements and proteins with the severity index of RA showed the following correlation coefficients: serum iron (0.35), copper (0.33), zinc (-0.42), ferritin (0.53) , hemoglobin (-0.37) and albumin (-0.66). **Conclusion**: These findings clearly indicated significant alterations in the levels of trace elements and proteins in the blood of RA patients. Low levels of iron and zinc and elevated levels of copper appeared to be associated with the risk of RA. The role of trace elements can be investigated further in the pathogenesis of RA and to test the efficacy of chelation/supplementation therapy for the treatment of RA.

B-300

Development of a New Biochip Based Immunoassay for the Measurement of Total 25-hydroxyvitamin D in Serum and the Accurate Classification of Vitamin D Status

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Background: 25hydroxyvitamin D [25(OH)D] concentration is directly related to the storage of vitamin D in the body and is the most widely used indicator of vitamin D status. Accurate measurement of 25(OH)D has well documented clinical implications for the diagnosis of vitamin D deficiency associated with musculoskeletal health and severe liver and kidney disease. More recent studies have shown that Vitamin D deficiency also has important implications in chronic illnesses including cancer and cardiovascular diseases. As a result of the emerging agreement on the implication of severe vitamin D deficiency on people 's health, there is an increasing need to assess vitamin D status in individual patients. The aim of this study was to evaluate a new biochip based immunoassay for the measurement of total 25(OH)D in serum on the fully automated, Evidence Evolution analyser. The immunoassay results were compared with a LC-MS/MS method, traceable to NIST Standard Reference Material STM 972 **Methods:** A direct competitive chemiluminescent immunoassay on a biochip platform and applied to the fully automated Evidence Evolution was utilized. Assay sensitivity was determined as functional sensitivity in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2. Repeatability was determined following CLSI protocol EP05-A3: 2 runs per day in duplication for 20 days (n=80). A method comparison study was conducted by analysing 20 serum samples from healthy individuals using the biochip based immunoassay, a LCMS/MS method and another commercially available immunoassay platform, the results were expressed as a % agreement, after being classified against established guidelines for each system. **Results:** The functional sensitivity evaluation showed sensitivity of 13.00 nmol/L. Repeatability was expressed as CV (%) for samples at the following concentrations; 45.5, 52.1, 76.7 nmol/L and was 12.8%, 10.3% and 12.9% respectively. In terms of the clinical classification of patient samples, the Evidence Evolution platform and LCMS/MS agreed on the classification of 20 samples out of 20 (100% agreement). The other commercially available immunoassay platform and LCMS/MS had an agreement on the classification of just 1 sample (5% agreement). **Conclusion:** The results show that this new biochip based immunoassay for the determination of 25(OH)D in serum and applied to the fully automated Evidence Evolution, presents optimal analytical performance and compares very favourably with LCMS/MS (100% agreement) in the classification of vitamin D status of serum samples. The Evidence Evolution is a high throughput analyser with random access and STAT capabilities. This platform is therefore a valuable and reliable analytical tool for the measurement of 25(OH)D.

B-301

Assessing diagnostic accuracy of serum Holotranscobalamine (Active-B12) in comparison with other markers of vitaminB12 deficiency.

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Background: Vitamin B12 deficiency has a prevalence of 15 - 40% in India and serum total vitaminB12 is the most commonly used test in its diagnosis. Total vitamin B12 exists in two bound forms: (i) bound to haptocorrin to form holohaptocorrin (70%-80%), (ii) bound to transcobalamin to form Holotranscobalamine (holoTC) (20%-30%). Body cells can only take up vitamin B12 in the form of holoTC. Therefore, measuring holoTC is more reflective of vitamin B12 status than measuring total vitamin B12 only. **Aim:** To assess the diagnostic accuracy of serum holoTC in comparison with total Vitamin B12 and total Homocystine as indicator of serum Vitamin B12 status. **Materials and methods:** 217 human subjects were assessed of which 99 were males and 118 were females ranging from 17 to 83 years of age. They were divided into 3 groups: (i) Vitamin B12 deficient (n= 70) with total vitamin B12 levels <200 pg/ml, (ii) borderline deficient (n=100) with levels 200 -350 pg/ml and (iii) sufficient group (n=47) with normal levels (>350 pg/ml). Markers of Vitamin B12 deficiency assessed were serum active vitamin B12, total Homocystine, Mean Corpuscular Volume (MCV), Folate, heamoglobin and creatinine for renal function. The samples were analysed using Siemens Advia Centaur Xpi. **Results**: Among the deficient group (n=70) most cases (14.3%) had low total vitamin B12 levels, low active Vitamin B12 but normal homocystine levels. 12.8% had low total B12 but normal active vitamin B12 levels and normal homocystine. 4 cases (5.7%) had low total vitamin B12, normal active vitamin B12 and elevated homocystine. However, among borderline deficient group (n=100) 14% had borderline total vitamin B12, low active vitamin B12 and elevated homocystine. While most cases (17%) had borderline total vitamin B12, low active vitamin B12 and normal homocystine. 15% had borderline total vitamin B12, normal active vitamin B12 and elevated homocystine . Also among the normal group (n= 47) only one case had low active vitamin B12 levels. Additionally, elevated MCV levels were found in 15.7% of the deficient group and 8.3% in the borderline group. **Conclusion:** Our study findings suggest that using combination tests of total B12, active B12 and plasma homocystine is required for improving diagnostic accuracy of Vitamin B12 deficiency.

B-302

VitaScan: Fast, portable, and low cost nutrition deficiency test

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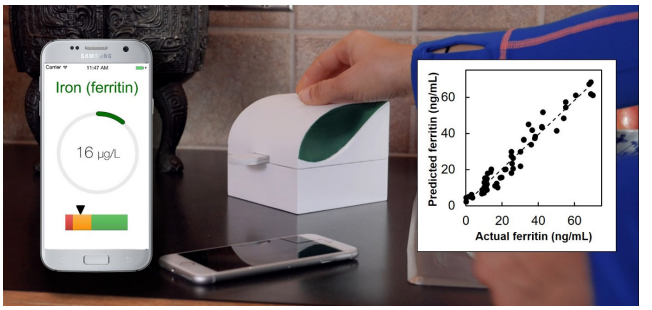
Background: Malnutrition is one of the most significant challenges in global health. For example, 2 billion people in the world are anemic, which is largely caused by iron deficiency and contributes to 20% of all maternal deaths. Existing bottlenecks include the limited access to tests and the inability to monitor people's health and provide feedback. Mobile diagnostic technologies largely have not reached technical requirements in speed, precision, and cost to viably address this problem. We present the solution through our rapid, low cost, and portable platform for quantifying nutrition biomarkers. Our initial focus is iron deficiency, and here we demonstrate strong analytical agreement between laboratory results and our ferritin assay, which is the strongest indicator of a person's iron status. **Methods:** Our platform technology incorporates simple sample processing, unique lateral flow design, and novel test imaging and analysis to enable quantitative determination of nutrition biomarkers at picomolar concentrations from a finger stick of blood. We demonstrate this with our disposable ferritin assay. A 40µL drop of finger stick blood is dispensed onto the assay, followed by running buffer. Optical contrast agents in the assay produce test and control lines at signal intensities relative to the native ferritin concentration in the blood. The assay cartridge is inserted into our dedicated reader platform, which analyzes the test and control lines to produce a quantitative result in under 10 minutes. **Results:** Our ferritin assay was tested against the Siemens Immulite ferritin lab test on 55 human samples. Samples showed linearity across the entire range of tested concentrations from 0 to 70 µg/L, producing an R^2 value of 0.95 and CV of 0.15. **Conclusion:** Our ferritin assay has demonstrated accuracy compared to laboratory instrumentation while showing superiority in portability, cost, and ease of use.



B-303

Measurements of blood trace elements in patients undergoing online hemodiafiltration

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Background: Essential trace elements play key roles in multiple biological systems, and hemodialysis patients are at risk for deficiency of essential trace elements. Blood levels of trace elements were previously measured in patients undergoing conventional hemodialysis, but there has been no data about those with online hemodiafiltration (online HDF). The aim of the study was to measure the serum concentration of copper (Cu), zinc (Zn), selenium (Se), and manganese (Mn) in patients with end-stage renal disease undergoing online HDF in outpatient dialysis clinic. Methods: A total of 28 patients (mean age 70.2 years, male 89%) were included. All were Korean. Blood samples were collected before and after one hemodialysis session, and serum concentrations of 4 trace elements were simultaneously measured by inductively coupled plasma mass spectrometry (PerkinElmer NexION 350D ICP-MS). Patients' clinical and recent laboratory data were also collected from medical records. Results: After the hemodialysis session, concentrations of all trace elements were significantly increased than the pre-hemodialysis levels. Mean post-hemodialysis levels were increased by 13.4% for Zn, 10.1% for Cu, and 8.1% for Se than pre-hemodialysis levels. Mn remarkably increased after session in 7 patients, and the remaining patients showed mean increase of 0.46 µg/L. Se and Zn deficiency were observed in 71% and 36% of the study participants with median 83.6 µg/L (reference range, 93-150 µg/L) and 70.8 µg/dL (66-110 µg/dL). Conclusion: The preliminary data suggest that the patients undergoing online HDF are also at increased risk of trace elements deficiency, especially for Se.

B-304

A quantitative method for magnesium measurement in red blood cells by ICP-MS

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Background: Magnesium is a mineral that is important for bone development, muscle contraction, nerve function and energy production. It is the fourth most abundant cation in the body with total content of about 25 g. The majority of magnesium are stored in the bone and inside cells with only ~ 1 % is present extracellular within the body; therefore, the measurement of plasma/serum magnesium concentrations correlates poorly with total body magnesium level. Hence, we developed an ICP-MS assay to accurately measure the intracellular magnesium level in the red blood cells. **Methods:** This method was developed on a Thermo Fisher X Series 2 ICP-MS with a collision/reaction cell controlled by PlasmaLab (v. 2.6.2.337). Scandium was used as the internal standard, and the total assay time is 1 min. The calibration standards were prepared at 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 mg/dL in 0.5 % nitric acid. Whole blood was first spin down for 5 min at 4000 rpm. The collected red blood cells were then diluted 1:100 with 0.5 % nitric acid and directly injected to MS for analysis. **Results:** The use of 0.5 % nitric acid as the artificial matrix for building calibration curve was verified in 10 individual patient samples through a mixing study. No interference was observed from exogenous compounds such as carbon (20,000 µg/dL), iron (25,000 µg/dL), sodium (65,000 µg/dL), calcium (30,000 µg/dL), chloride (200,000 µg/dL) and MRI contract mix (10,000 µg/dL of iodide, gadolinium and barium). Analytical measuring range was determined to be between 0.7 mg/dL to 29.3 mg/dL with analytical recovery ranging from 99.5 % to 118.8 %. No significant carryover was observed from a sample at 40.0 mg/dL. CV for total precision and intra-assay preci-

sion was between 3.1 % to 4.2 % and 1.5 % to 3.5 %, respectively at three concentration levels of 3.2 mg/dL, 4.6 mg/dL and 6.0 mg/dL (n = 30 each). This method was compared to another ICP-MS method offered at an independent lab using 44 patient samples with a concentration range of 3.0-6.6 mg/dL; and the Deming regression showed R of 0.8837, slope of 0.937, intercept of 0.35 and overall bias of 1.55 %. RBC magnesium was stable for 1 week at ambient, 1 month at refrigerated and unstable at frozen. Whole blood samples should be separated from cells within 4 h. **Conclusion:** This new ICP-MS method for quantifying magnesium in red blood cells is highly sensitive and accurate. It has been validated for clinical use.

B-305

Iodine- and Gadolinium-based Magnetic Resonance Imaging Contrast Agents Interfere with Inductively Coupled Plasma Tandem Mass Spectrometry-based Trace Element Testing

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Background: Gadolinium (Gd)- and iodine (I)-based magnetic resonance imaging (MRI) contrasting agents have been reported to interfere with the inductively coupled plasma mass spectrometry-based (ICP-MS) quantitation of trace elements in human matrices. However, limited comprehensive literature describing the effect of these agents on the inductively coupled plasma tandem mass spectrometry-based (ICP-MS/MS) quantitative testing of plasma and urine trace elements is available. ***Objective:*** Evaluate potential interference from the MRI contrast agents: gadobutrol (Gadovist™); gadodiamide (Omniscan™); iohexol (Omnipaque™); ioversol (Optiray™); and iodixanol (Visipaque™) on plasma and urine trace element quantitation by ICP-MS/MS. ***Methods:*** Plasma and urine specimen pools were respectively created from *N*=10 specimens. *N*=5 unique aliquots of each matrix pool were then created with subsequent aliquots respectively spiked with 0, 0.05, 0.1, 0.5, 1, 5, 10, 15 and 20 µg·mL^{-1} of an individual MRI contrast agent. Prior to their introduction to an Agilent 8800 ICP-MS/MS, all plasma and urine samples were diluted in a solution containing nitric acid and ethanol. Aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr), thallium (Tl), tin (Sn), uranium (Ur), vanadium (V) and zinc (Zn) were quantified in both plasma and urine. All specimens were analyzed in duplicate. Interference from the MRI contract agent was considered significant if the percentage difference between the experimental and expected trace element concentration exceeded the tests defined total allowable error at any of the tested MRI contrast agent concentrations. ***Results:*** Iodine contamination of the ICP-MS/MS instrument occurred following the analysis of any specimen with an iohexol, ioversol or iodixanol concentration ≥5 µg·mL^{-1}. Maintenance was required to remove this contaminant and eliminate the high iodine background ion count within this ICP-MS/MS system. MRI contrast agents causing significant interference with plasma trace elements: gadobutrol (Al, I and Hg); gadodiamide (Al, Be, B, Co, Cu, Hg, Mo and Zn); iohexol (Cr, I and Sn); ioversol (B, Co, Cu, I, Mo, Sn, V and Zn); and iodixanol (As, Co, Cu, I, Sn and V). MRI contrast agents causing significant interference with urine trace elements: gadobutrol (Al, As, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, Tl and Zn); gadodiamide (Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, Tl and Zn); iohexol (Al, I and Tl); ioversol (Al, I and Tl); and iodixanol (Al, As and I). All other plasma and urine trace element tests were not interfered with by the MRI contrast agents at the concentrations tested. ***Conclusions:*** Plasma and urine specimens containing iohexol, ioversol or iodixanol can introduce significant iodine contamination to an ICP-MS/MS system. If I- and Gd-based MRI contrast agents are administered to a patient, plasma and urine specimen collection for trace element testing should be delayed due to the potential for significant analytical interference with multiple analytes.

B-306

Urine cadmium analysis - Is molybdenum oxide a concern?

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Introduction:
Cadmium is a toxic and carcinogenic metal associated with increased risk of cancer and cardiovascular diseases in prolonged extreme exposure. Urine Cadmium is commonly measured in individual either with clinical presentations suggesting acute toxicity or as compliance to regulatory requirement of safe biological exposure indices. SGH Clinical Biochemistry offers the Urine Cadmium test as routine clinical services using Inductively Coupled Plasma Mass Spectrometry. It is widely recognized that the presence of Molybdenum Oxide may introduce false positive to Cadmium analysis. This paper describes the use and compares the mathematical correction against Dynamic Reaction Cell in removing of Molybdenum polyatomic interference in Cadmium Urine analysis.
Methods:
2 solutions containing; i) Molybdenum standard (100ppb) and ii) blank using acid diluent were prepared and measured for Cadmium amu111 and Molybdenum amu95 signal intensity counts. Fresh gravimetric-preparation of Cadmium standards (6 points; 20xdilution of Multi Calibration Standards) were prepared and analyzed for calibration of Cadmium test on Perkin Elmer Elan DRC II, using 2 different applications of standard mode and dynamic reaction cell (oxygen gas at 2.0ml/min). Recovery studies were performed on samples spiked with 100ppb of Molybdenum and 0.4ppb of Cadmium.
Results:
The first method uses the mathematical equation to correct the interfering Molybdenum from the Cadmium. The factor (F) is calculated by [(111 Mo - 111 blank)/(95 Mo - 95 blank)] to be 0.04 and applied to all analyzed sample. The second method uses reaction gas oxygen to eliminate the bulkier polyatomic interference of molybdenum oxide in a dynamic reaction cell. The recovery of Cadmium with mathematical calculation and DRC mode is calculated to be 94 to 104 % and 112 to 114%, respectively.
Conclusion:
This study demonstrated that mathematical equation is more effective in correcting the interference of Molybdenum in urine Cadmium measurement using PerkinElmer Elan DRCII. Molybdenum oxide is a known polyatomic interference of cadmium analysis and must be corrected to minimize false positive result.

B-307

Significant Loss of Blood Amino Acids and Free Carnitine in Newborns Receiving Continuous Renal Replacement Therapy (CRRT)

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Background: Newborns with acute kidney injury (AKI) or end-stage renal disease (ESRD) often receive prolonged CRRT when the early initiation of peritoneal dialysis is either contraindicated or unable to be performed. These patients often receive total parenteral nutrition (TPN) to meet their nutritional goals. A little to no information exists on the loss of blood amino acids (AA) and free carnitine during CRRT in these patients. The objective of this study was to determine the amino acids and free carnitine losses in newborns receiving prolonged CRRT and TPN.
Methods: Three newborns who received prolonged (> 2 weeks) CRRT and TPN were included in the study. Blood and CRRT effluent were simultaneously collected from these patients. The effluent specimens were collected over 8-12 hours and the results were extrapolated to 24 hrs. Plasma was separated from blood for the analysis of 30 amino acids and free carnitine. Amino acids in plasma and CRRT effluent were analyzed using amino acid analyzer which uses ion-exchange chromatography and post-column ninhydrin derivatization (Biochrom System). Free carnitine was determined by HPLC-tandem mass spectrometry (HPLC-MS/MS) using flow injection, electrospray ionization and precursor ion scan. The total amount of amino acids and carnitine received by each patient was calculated from the amino acids concentrate and carnitine added to TPN solution. The sieving coefficients (SQ) for each measured amino acid and carnitine was determined, while the losses of amino acids and carnitine losses were calculated as mg/day, and as percentage of the intake.
Results: The amino acid profile of 30 amino acids was determined in 3 patients and free carnitine was performed in 2 of these patients. The CRRT clearance ranged from 68-115 mL/kg/hr (1.4 -3.2 L/1.73m2/hr). Interestingly there was significantly different SQs for different categories of amino acids. The SQ for all essential amino acids was >80% with loses of 10-20% of their intake. SQ for acidic AAs (glutamic acid and aspartic acid) was <40% with <5% of their intake. SQ for cystine exceeded 100% and all patients had a low plasma cystine level. SQ for carnitine was >84%, and carnitine losses were 80% of its intake. This led to decreased free plasma carnitine concentrations.
Conclusions: CRRT leads to significant loss of many amino acids and free carnitine. The amino acids losses during CRRT are not uniform and can result in significantly low concentrations of certain blood amino acids. Additional studies are needed to determine if patients receiving CRRT require special amino acids formulations as part of TPN to account for these amino acids losses. As carnitine is rapidly and freely filtered during CRRT, appropriate increase in carnitine dose is necessary to avoid its deficiency.

B-308

Vitamin D Status and Glycemic Control: Implications for Pre- and Postmenopausal Women

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Background
Emerging evidence indicates that vitamin D deficiency is associated with several chronic diseases. Vitamin D levels has been implicated with abnormal glycemic control and estrogen levels. Aging and the subsequent decline in oestrogen during menopausal stages promotes hypovitaminosis D. Nonetheless, the interaction between vitamin D, menopause, lifestyle and T2DM requires extensive study. This study provides the first evidence of vitamin D status among pre- and postmenopausal T2DM in the Ghanaian population, determined the association between vitamin D status and glycemic control and also assessed the influence of lifestyle habits on hypovitaminosis D.
Methods
In a cross-sectional study conducted at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. Structured questionnaires were administered to 192 consenting pre- and postmenopausal T2DM women with more than 6months disease duration. Their blood samples were collected for estimation of (25OH) D and Insulin using ELISA. Fasting blood glucose (FBG), Lipid profile, Glycated haemoglobin (HbA1c) and calcium were measured enzymatically. Statistical analyses were performed using Graphpad Prism 6.
Results
The prevalence of vitamin D inadequacy was 92.2%. Hypovitaminosis D was more prevalent among the postmenopausal T2DM (63.8% vs 58.2%). Hypovitaminosis D significantly associated with Insulin [R^2=0.01760,p=0.0008], HbA1c [R^2=0.3709,p=<0.0001], FBG [R^2=0.3465,p=0.0001] in only the postmenopausal women. A higher risk of developing vitamin D deficiency was associated with unemployment (aOR=1.612, 95% CI (0.828-3.138), p = 0.160), being both uneducated (1.095 95% CI (0.094-12.800), p = 0.943) and educated 2.236 95% CI (0.222-22.529), p= 0.495) having diabetes mellitus for > 5 years (aOR = 1.842, 95% CI (0.926-3.664), p = 0.082), higher WHR (aOR = 1.419, 95% CI (0.594-3.392), p = 0.431) and WHtR (aOR = 1.336, 95% CI (0.723-2.468), p = 0.355)
Conclusion
Vitamin D deficiency is prevalent in pre- and postmenopausal T2DM but higher among postmenopausal women. Adequate vitamin D levels in both groups was associated with improved glucose control while hypovitaminosis D in the postmenopausal women was related to poorer glucose control. Vitamin D screening should be incorporated into the management plan for T2DM to serve as an early tool for prevention of Vitamin D deficiency.
Keywords: Vitamin D, diabetes, postmenopausal women, insulin resistance, glycemic control.

B-309

Analysis of biomarkers of calcium metabolism in bariatric surgery patients

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Background: Bariatric surgery patients have an increase in bone metabolism after surgery. One hypothesis is that their rapid weight loss results in skeletal unloading, reducing the bone mass density (BMD). However, studies have found that bariatric patients are at high risk for vitamin D deficiency and secondary hyperparathyroidism which lead to pathological changes in BMD. Therefore, it is important to monitor biomarkers of calcium metabolism, such as PTH, vitamin D, and calcium. We examined how these three markers were related in bariatric surgery patients over time with multilevel mixed-effects models.
Methods: A retrospective chart review of 358 sleeve gastrectomy (SG) and 110 Roux-en-Y gastric bypass (RYGB) patients who had recorded vitamin levels and a bariatric procedure from April of 2012 to April of 2016 was performed. Data were collected for vitamin D (Abbott Architect), PTH (Siemens Centaur), albumin, calcium (corrected for albumin, Siemens Vista), estimated GFR, and the presence of diabetes and hypertension. Mean levels, deficiencies in vitamin D, and elevations in PTH were analyzed and grouped according to pre-operative, ≤1 year post-operative, and >1 year post-operative period. The cut-off for vitamin D deficiency was <20 ng/mL, and the reference range for PTH was 12-72 pg/mL. Statistical analysis was performed using Stata. Multilevel mixed-effects quadratic models were used to determine if the change in analyte level was significant over time (months) and related to changes in the other biomarkers. We investigated what factors contributed to elevated PTH in our bariatric patients by examining the effects of vitamin D deficiency, surgery type, and chronic kidney disease. The study was approved by our IRB.
Results: Vitamin D deficiencies decreased the first year after surgery, from approximately 27% pre-operative deficiencies to 5.2% in SG and 11.5% in RYGB patients. In the ≥1 year post-operative period, SG participants had 13.4% and RYGB had 20.3% vitamin D deficiencies. Elevated PTH remained constant at approximately 20% of SG patients; however, 45% of RYGB patients had at least one elevated PTH level in the ≥1 year post-operative period. Mean PTH levels for RYGB patients were 52 ± 27 pg/mL at baseline (pre-op) and 73 ± 29 pg/mL at greater than 1 year post-surgery. The multilevel models showed some predictable trends in the data, such as increasing levels of vitamin D and corrected calcium were inversely associated with levels of PTH. Vitamin D deficiency was significantly associated with an average increase of 16 pg/mL of PTH (p<0.005). For the effect of surgery type, RYGB patients had an increase of 11 pg/mL for PTH and decrease of 4 ng/mL for vitamin D when compared to SG patients (p<0.05). Additionally, CKD stages had an effect on PTH levels: baseline was no CKD or stage 1, 5 pg/mL increase for stage 2 (not significant), 19 pg/mL increase for stage 3 (p<0.005), and 42 pg/mL increase for stage 4 (p<0.05).
Conclusions: Risk factors associated with increased PTH in our bariatric patients were vitamin D deficiency, RYGB surgery type, and CKD status. Patients with these risk factors may need more aggressive management to prevent decreased BMD.

Wednesday, August 1, 2018

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

Maternal-Fetal, Pediatrics, and Fetal Clinical Chemistry

B-310

Fetal risk assessment in high-risk Brazilian first trimester pregnant: a retrospective observational study.

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Background: Aneuploidies are the most common genetic abnormalities, which can be detected during prenatal care. Trisomies involving chromosomes 13 (Patau syndrome), 18 (Edward's syndrome) and 21 (Down's syndrome) are the most frequently aneuploidies identified by karyotyping or high-risk serum markers. In addition, open neural tube defects (NTDs) is also investigated in fetal risk assessment. Recently, prenatal screening for these disorders are based in tests which evaluate the concentration of serum markers and combine them with information about the pregnant health, such as age, weight, and gestational age. Together, these data estimate the fetal risk to develop the disorders above. The Multiples of the Median (MoM) values are calculated using a database resource of low-risk pregnancies with adjusted data for gestational age, insulin-dependent diabetes mellitus, multiple pregnancy, *in-vitro* fertilization, smoking status and systematic differences between laboratories and assay reagents. **Objective**: The aim of this study was to describe the high-risk gestational profile of first trimester pregnant (between 10 and 13 weeks of pregnancy) from samples collected at Hermes Pardini Institute between 2016 and 2018. **Methods:** The AutoDELFIA™ hAFP/Free hCGß (PerkinElmer, US) and AutoDELFIA® PAPP-A Kit (PerkinElmer, US) were used for detection of the total and free ß human chorionic gonadotrophin (hCG) and pregnancy associated plasma protein A (PAPP-A) levels, respectively. All the assays were performed following manufacturer's instructions. The ALPHA program (Logical Medical Systems Limited, UK) was used to calculate the risk of pregnancies with trisomies involving chromosomes 13, 18 and 21, and open neural tube defects (NTDs) in first trimester of pregnancy. In addition, this software considers the serum markers levels besides the value of nuchal translucency (NT). **Results:** One total of 10,301 patients data were analyzed between 2016 and 2018 and the median of maternal age was 32 years (51.6% of cases). Among them, 88.1% of cases were negatives for Down's syndrome and NTD, and 11.4% were positive for the disorders investigated: 9.9%, 0.8% and 0.7% showed increased risk for Down's syndrome, trisomy 18 and for others fetal risk disorders development, respectively. It were not reported risk for trisomy 13. The results observed for increased risk of the disorders at pregnant age were 2.0%, 9.7%, 49.8%, and 38.5% for 10, 11, 12, and 13 weeks, respectively. **Conclusions**: Currently, the assessment of fetal risk is indicated only for high-risk pregnancies.According to the Brazilian Ministry of Health and National Institutes of Health of US, advanced maternal age (>35 years) is one of the criteria to characterize one high-risk pregnancy.. Despite this, it was observed that the mean age of the cases evaluated was 32 years, indicating that one sample of Brazilian women had high-risk pregnancy with earlier age. Therefore, the assessment of fetal risk could be a good predictor for high-risk pregnancies, since it is less invasive than the gold standard techniques such as karyotyping, and seems to be more assertive than image tests alone.

B-311

The effect of the enzyme replacement therapy on the liver function tests in children with c Disease (GD)

H. A. Abdulhussain[1], H. A. Abdulamir[2], H. T. Noaman[3], H. S. Arif[4]. *[1]Ain Al-Tamer Hospital, Karbala, Iraq, [2]College of Pharmacy-Al-Nahrain University, Baghdad, Iraq, [3]College of Medicine, AL-Mustansiriyah University, Baghdad, Iraq, [4]Pediatric Department College of Medicine, Al-Nahrain University, Baghdad, Iraq*

Background: GD is an inherited autosomal recessive disease. It is most common in the Ashkenazi Jewish population. Many biomarkers might be involved in the etiology, pathogenesis, diagnosis and prognosis of this disease in children. Most of them are related to complications due to an involvement of many organs such as liver due to lack of the glucocerebrosidase enzyme. **Objectives:** to investigate the role of assisting liver function tests in the diagnosis and monitoring Gaucher patients receiving enzyme replacement therapy. **Methods:** A case control study was done on 67 children (male & female) age range from 1-15 years who had GD recruited from Pediatric Department and Unit of rare disease at Al Imamain Al-Kathemeaain medical city, Gastroenterology and Hepatology Teaching Hospital, Children Welfair Hospital Consultation Clinic and Central Child's Teaching Hospital. The levels of ALT, AST, ALP, total bilirubin and total protein were measured in the samples of 67 Gaucher patients who were categorized as newly diagnosed un treated patients (n=9), patients receiving ERT for 3-6 months (n=18) 6-12 months (n=20) and patients receiving ERT for more than one year (n=20) and compared with twenty newly comparable age-matched control subjects. The practical part of the study was conducted in the Department of Chemistry and Biochemistry, College of Medicine, University of Al-Nahrain from December 2016 to March 2017. The levels of these biomarkers were determined by colorimetric methods according to manufacturer instruction. **Results:** The data indicated that the mean± standard deviation (SD) levels of ALP in whole Gaucher patients (210.27 ± 61.21 U/L) were significantly higher ($p<0.05$) than that of age-matched controls (163.17 ± 49.34 U/L, respectively) while the level of total protein in patients (6.29 ± 0.73 g/dl) were significantly lower ($p<0.05$) than that of age-matched controls (6.81 ± 0.32 g/dl). On the other hand, non-significant differences were illustrated in the levels of ALT, AST and total bilirubin. These parameters were remarkably associated with the period of receiving treatment with ERT that indicated by the negative significant ($p<0.05$) correlations between the levels of AST (r=-0.476; $p<0.001$), ALT (r=-0.448; $p<0.001$), ALP (r=-0.394; $p<0.001$) and total bilirubin (r=-0.343; p=0.001) and period of receiving treatment and positive significant ($p<0.05$) correlations between the levels of total protein (r= 0.484; $p<0.001$) and the period of receiving treatment. The effect of ERT also revealed be the results obtained by ANOVA test that indicate significant ($p<0.05$) differences among the patients subgroups in the levels of ALT, AST, total bilirubin and total protein. I I **Conclusions:** Liver function tests showed to have a diagnostic value in newly diagnosed untreated patients with diversity in their response to the treatments that limit their role in the monitoring of the treatment. III

B-312

Reformulation of the Roche total bilirubin Gen3 reagent did not affect the relationship between BiliChek transcutaneous and Roche total serum bilirubin

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Background: American Academy of Pediatrics guidelines recommend transcutaneous (TcB) or total serum (TSB) bilirubin measurement for many newborns. In our institution all term infants are screened with TcB prior to nursery discharge. TcB is plotted with post-natal age in hours to determine risk for severe hyperbilirubinemia using the Bhutani nomogram. Infants with high intermediate risk (HIR) or high risk (HR) TcB values have confirmatory TSB values determined, with all further treatment based upon TSB. The use of different bilirubin laboratory methods or changes in the calibration or formulation of the laboratory methods may impact the relationship between TcB and TSB, and thus the efficacy of TcB screening. In 2014 Roche Diagnostics announced a reformulation of the total bilirubin reagent that could potentially affect bilirubin results. The objective of this study was to determine whether reformulation of the Roche total serum bilirubin reagent affected the relationship between TcB and TSB. **Methods**: TcB results of all neonates in the level 1 newborn nursery with a subsequent TSB measurement within 1 hour were reviewed; during a period of six months before and after the conversion from the old Roche total bilirubin (BILTS) reagent to the new Roche Gen3 bilirubin assay. TSB was measured on a Roche Cobas c501 analyzer (Roche Diagnostics, IN). TcB measurements were performed using the BiliChek transcutaneous bilirubin monitor device (Respironics, Marietta GA), and calibrated with a disposable tip (BiliCal). Distribution of TSB results, and TcB minus TSB bias, were compared before and after the introduction of the reformulated Roche total bilirubin Gen3 assay. Median and interquartile range (IQR) TSB values, and median and IQR bias (TcB minus TSB) were calculated. A statistical difference between median values of TSB and median bias were assessed using Man-Whitney test. **Results**: A total of 301 paired (obtained within one hour of each other) TcB and TSB results were obtained, 172 before and 129 after implementation of the reformulated Roche Gen3 reagent. The distribution of TSB results, before and after the implementation, showed a similar pattern. TSB median (IQR) concentration was 7.8 (6.8-8.7) mg/dL before and 7.6 (6.7-8.4) mg/dL after implementation of the reformulated reagent (p=0.1373). Median (IQR) bias between TcB and TSB was 2.9 (2.2-3.7) mg/dL

before the reformulated reagent was implemented; and did not change at 2.9 (2.1-3.9) mg/dL after the reformulated reagent was implemented (p=0.8242). **Conclusion**: Reformulation of the Roche total bilirubin Gen3 assay did not affect the relationship between BiliChek transcutaneous and serum bilirubin; and thus no changes were needed to the neonatal TcB screening protocol as a result of the modified bilirubin reagent.

B-313

Association of Fibroblast Growth Factor 21 Plasma Levels with Infection in Neonates: Preliminary Results

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Background: Infections remain one of the leading causes of morbidity and mortality in neonatal age and may also have severe long-term consequences. Identification of new or complementary biomarkers of neonatal infection or sepsis is of great importance. Fibroblast growth factor 21 (FGF21) is a member of the FGF superfamily, consisting of FGF19, FGF21, and FGF23. FGF21 has emerged as a key regulator in the metabolism of glucose and lipids. A possible role of FGF21 in sepsis has been suggested by the observation of increased circulating levels of this hormone during experimental sepsis in mice. Moreover, its administration has a protective effect from the toxicity of lipopolysaccharide (LPS) and sepsis. FGF21 can also reduce the severity of cerulein-induced pancreatitis in mice, further indicating that FGF21 could modulate inflammation. These findings highlight the possible role of FGF21 as a biomarker and a therapeutic tool in mice with sepsis and an inflammatory state. As the involvement of FGF21 in neonatal infection is not known yet we aimed to explore the clinical value of circulating FGF21 levels as biomarker of neonatal infection. **Methods:** Seventy-seven full-term neonates were included in the study: of them 25 with febrile bacterial infection and 52 without any infections. Along with hematologic and blood chemistry parameters, plasma levels FGF-21 were determined by means of an immunoenzymatic technique. **Results:** Plasma FGF21 levels were significantly higher in neonates with infection compared to controls ($p<0.001$). FGF21 levels on admission correlated significantly with serum CRP levels ($r_s=0.487$, $p=0.01$) and also with plasma glucose ($r_s=0.446$, $p<0.05$) and triglyceride levels ($r_s=0.419$, $p<0.05$). In multiple regression analysis, the correlation between FGF21 and CRP levels remained significant after adjustment for glucose or triglyceride levels. Receiver operating characteristic analysis of FGF21 levels resulted in significant areas under the curve (AUC) for detecting infected neonates on admission (AUC=0.965, $p<0.001$). **Conclusions:** Circulating FGF21 levels are increased at the acute phase of neonatal infection possibly reflecting and/or participating in the inflammatory process, and correlated also with metabolic parameters. Thus as sepsis is associated with insulin resistance, we can also hypothesize that the increase in plasma FGF21 observed in the neonates with infection might also be due, at least partly, to insulin resistance. Insulin resistance in sepsis is due to a decreased effect of insulin, but also reflects an imbalance between insulin and its counter-regulatory hormones such as cortisol, glucagon, growth hormone and catecholamines. FGF21 may be used as an early marker of neonatal infection, however, prior to its clinical usefulness, this protein must undergo through rigorous validation in multiple cohorts.

B-314

Acetylserotonin O-Methyltransferase (ASMT)/rs4446909 Polymorphism in Iraqi Autistic Children

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Background: The genetic causes in addition to hormonal, neurological, and immunological basis for autism is still not fully understood, and the role of the interaction among neuro-inflammation, genetic, immunological mediators and neurotransmission impairment needs to be clearer. ASMT is an enzyme that involved in the synthesis of melatonin which is assumed to have a possible role in autism pathogenesis. **Objectives:** to explore the potential effect of the acetylserotonin O-methyltransferase (ASMT)/rs4446909 polymorphism on the risk of autism and to test the possible association between this single nucleotide polymorphism (SNP) with the severity of social and cognitive dysfunctions in male children with autism in order to assess the possibility of using this SNP in the prognosis of autism severity. **Methods:** A case control study was carried out in the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad- Iraq and Forensic DNA Research and Training Center/Al-Nahrain University\Baghdad\Iraq. The study was done on 60 male patients with autism who were recruited from Department of Pediatrics at Al-Sader Hospital, Baghdad-Iraq between November 2014 and April 2015. DNA obtained from the Erythrocytes of autistic male patients who were categorized as mild (n=20), moderate (n=20) and severe (n=20) according to diagnostic and statistical manual of mental disorders and compared with Thirty age-matched control subjects. The genetic polymorphisms ASMT/rs4446909 were detected by polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP) method. **Results:**
It was demonstrated that there were non-significant differences in ASMT/rs4446909 polymorphism between the whole autistic group patients and control groups. On the other hand, the study of ASMT/rs4446909 polymorphism revealed that severe autistic patients who carried G/G genotype showed a significant higher autism risk when compared to individuals who carried the A/G genotype. **Conclusion:** ASMT/rs4446909 polymorphism is not found to be associated with autism in the studied children whereas an association between the severity of autism and studied genotype were illustrated. This may pinpoint the involvement of this polymorphism in the pathogenesis of autism.

B-315

The Effect of the Enzyme Replacement Therapy on the Kidney Function Tests & Serum Electrolyte Levels in Children With Gaucher Disease

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Background: Gaucher disease is an inherited autosomal recessive disease. It is most common in the Ashkenazi Jewish population. Many biomarkers might be involved in the etiology, pathogenesis, diagnosis and prognosis of Gaucher disease (GD) in children. Most of them are related to complications due to an involvement of many organs such as liver, spleen and bones by this lysosomal storage disease that caused by a lack of the enzyme glucocerebrosidase. **Objectives:** to investigate the role of kidney function test and electrolytes (urea, creatinine, sodium and potassium) level in the monitoring of the response for the treatment used for patients with Gaucher's disease in follow-up manner **Methods:** A case control study was done on 67 children (32 male & 35 female) age range from 2-14 years (mean± SD; 5.3±2.9). The levels of sodium, potassium, urea and creatinine were measured in the samples of patients who were categorized as newly diagnosed untreated patients (n=9), patients receiving ERT for 3-6 months (n=18) 6-12 months (n=20) and patients receiving ERT for more than one year (n=20) and compared with twenty age-matched control subjects (9 male & 11 female) age range from 2-14 years (mean± SD; 5.55± 3.05). **Results:** The data indicated that the level of urea in GD patients (23.39 ± 4.71 mg/dl) was significantly higher than that of age-matched controls (17.5 ± 3.05 mg/dl). Non-significant differences were illustrated in the levels of sodium, potassium and creatinine. Negative significant ($p<0.05$) correlations were obtained between the levels of urea ($r= -0.752$; $p<0.001$) and creatinine ($r= -0.536$; $p<0.001$) with the period of receiving ERT. Additionally, ANOVA test also revealed significant ($p<0.05$) differences among the patients subgroups in the levels of urea and creatinine. Results obtained from Receiver Operating Characteristic (ROC) curve revealed that urea and creatinine showed a high area under the curve (AUC), sensitivity and specificity (0.939, 77.8% and 85% for urea and 0.978, 100% and 80% for creatinine respectively) in newly diagnosed GD patients in a comparison with control. **Conclusions**: the possibility of using urea and creatinine in the diagnosis and monitoring the effect of ERT on the GD patients.

B-316

CALIPER Pediatric Reference Intervals for Siemens Biochemical Assays on ADVIA XPT and Dimension EXL with LM Integrated Chemistry Systems

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Background: Reference intervals (RIs) are the central 95% of laboratory test results obtained from a cohort of healthy reference individuals. Accurate RIs are required for clinical interpretation of test results and diagnosis. However, several gaps exist in pediatric RIs due to the significant physiological changes that occur during pediatric development as well as difficulties involved in recruiting a large number of children and adolescents. CALIPER (Canadian Laboratory Initiative on Paediatric Reference Intervals) is a Canada-wide initiative to fill these gaps by establishing age- and sex-specific RIs on various clinical chemistry analyzers. The current study expands the CALIPER database by establishing age- and sex-specific RIs on two Siemens platforms: ADVIA Chemistry XPT and Dimension EXL with LM Integrated Chemistry Systems. **Methods:** A large cohort of healthy children and adolescents (<19 years old) who completed health questionnaire forms were recruited from GTA (Greater Toronto Area) and Hamilton regions as part of the CALIPER study and donated blood samples. Those with acute or chronic illnesses and/or recent medication use were removed from analysis. Serum samples of a total of 909 and 867 healthy participants were tested on ADVIA XPT (33 assays) and Dimension EXL (21 assays) systems, respectively. Analyte concentrations were visually inspected for age- and sex-based partitions, which were statistically confirmed using Harris and Boyd's statistics. Outliers were removed using Tukey or adjusted Tukey for parametric and nonparametric data, respectively. According to CSLI C28-A3 guidelines, age- and sex-specific 95% RIs, along with 90% confidence intervals, were calculated using either the nonparametric rank method ($n \geq 120$) or the robust method of Horn and Pesce ($40 \leq n < 120$). **Results:** Serum concentrations of several assays remained relatively constant within pediatric age range and similar between sexes, including C4, cholesterol, CRP, sodium, total iron binding capacity, and triglycerides. Other tests, such as alkaline phosphatase, enzymatic creatinine, lactate dehydrogenase, and total bilirubin, showed significant changes throughout pediatric age and differences were evident between males and females mostly after puberty. Furthermore, immunoglobulin G, total protein, and direct bilirubin and several others required age partitioning, but sex differences were not observed even after puberty. **Conclusion:** Age- and sex-specific RIs were established for a combined total of 54 assays on Siemens ADVIA XPT and Dimension EXL systems. These results will allow for a more accurate laboratory assessment of pediatric patients with the use of these two Siemens platforms in clinics and hospitals around the world. However, it is recommended that these reference values be verified, based on CLSI guidelines, using local pediatric samples and analyzers before clinical use.

B-317

Development of an Automated Assay for the Measurement of Free Beta Human Chorionic Gonadotropin (FBHCG) on the Siemens ADVIA Centaur XP Immunoassay System

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Background: Human chorionic gonadotropin (HCG) is secreted by placental tissue and serves to support the corpus luteum during the early weeks of pregnancy. HCG is composed of α and β subunits while the subunits can also occur in free forms. Serum free β-HCG assessment is reported to improve detection in first- and second-trimester prenatal screening for chromosomal anomalies. The efficiency of prenatal screening in first trimester using a combination of maternal age, serum free β-HCG, serum PAPP-A, and fetal nuchal translucency measurements might be significantly improved when compared to second-trimester screening. Using this approach, various investigators have reported detection rates for Down syndrome of 85-90% at a 5% false-positive rate.[1] **Method:** A chemiluminescent immunoassay for the detection of free β-HCG has been developed. The ADVIA Centaur® Free Beta Human Chorionic Gonadotropin (FBHCG) Assay† is intended for in vitro diagnostic use in the quantitative determination of the free β subunit of HCG in serum using the ADVIA Centaur XP Immunoassay System. Free β-HCG is bound to paramagnetic microparticles coated with anti-free β-HCG antibody and is then detected by an acridinium ester (NSP-DMAE)-labeled anti-free β-HCG antibody. Following incubation, wash, and magnetic separation steps, acidic and basic reagents are added. The resulting chemiluminescence is measured. Assay performance was evaluated for precision, linearity, limit of quantification (LOQ) and method comparison to B·R·A·H·M·S Free βhCG KRYPTOR. The method comparison study was performed per CLSI EP-09-A3 using 147 patient samples. A precision study was carried out over 20 days according to CLSI EP5-A3. Linearity and LOQ studies followed CLSI EP06-A and EP17-A2, respectively. Performance of the assay was also assessed against a list of potential interfering substances and cross-reactants, following CLSI-EP07-A2. **Results:** The reportable range of the assay is up to 200 IU/L without dilution, or up to 2000 IU/L with automated 1:10 dilution. Linearity has been demonstrated up to 200 IU/L. The limit of quantitation was 0.28 IU/L. The precision study had a within-lab CV of 2.9-4.8%. The method comparison of the assay to the B·R·A·H·M·S Free βhCG KRYPTOR returned a slope of 1.03 and intercept of 0.65 IU/L by Passing-Bablok regression and a Pearson coefficient (r) of 0.99. The assay demonstrated no significant interference from hemoglobin, conjugated and unconjugated bilirubin, triglycerides, biotin, cholesterol, protein albumin, gamma globulin, rheumatoid factor, and human anti-animal antibodies. The assay demonstrated no cross-reactivity with intact HCG, follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone. **Discussion and Conclusions:** The performance of the FBHCG assay on the Siemens ADVIA Centaur XP system has been assessed and the results show an accurate and precise method for the measurement of free β-HCG in human serum. **Reference:** 1. Shiefa S. et. al. Indian J Clin Biochem. 2013;28(1):3-12. †Underdevelopment.Notavailableforsale,anditsfutureavailabilitycannotbeguaranteed.

B-318

Clinical case report: Patient with ring 14 chromosome with no associated deletion presenting severe clinical

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Background: Ring 14 chromosome syndrome has a large number of associated abnormalities. The identification of these abnormalities may be essential to provide an early diagnosis and for the genetic counseling. The case report stated the importance of early clinical suspicion for monitoring the clinical evolution of individuals with Ring 14 chromosome syndrome. Resuming the clinical discussion of patients with this rare condition. Ring 14 chromosome syndrome is a rare condition, of which exact clinical identification is still limited. The objective of this study is to provide information and data about this rare condition and to ratify the importance of conventional cytogenetics in the diagnosis and genetic counseling. **Methods:** The focus of our study is a 1-year-old male patient, submitted to conventional karyotyping with clinical indication of developmental delay and difficult-to-treat focal epilepsy, microcephaly and facial dysmorphia. With peripheral blood material, two cell cultures of lymphocytes. From the obtained material was carried out the analysis, being karyotyped 50 metaphases. It has a complete chromosomal ring with no apparent loss of chromosomal material or a small terminal deletion (telomere loss) in all cells analyzed in pure lineage, without mosaicism - 46,XY,r(14)(p13q32). The analyzed patient presented clinically the characteristics correlated to the evidenced diagnosis **Results:** In the nucleus of Cytogenetics DASA S.A in 2017, 495 karyotypes with the clinical indication delay and/or deficiency in the development were analyzed. Inside these 495 karyotypes, the age range was 0 to 9 years. The cytogenetic study of this group had 98% of normal results and in 34% there were polymorphic variants of the population in general. Only 2% had an altered karyotype. Of these 2% who presented altered karyotype all had one or more clinical characteristics added to the delay in development. This data reinforces the importance of the clinical indication in conducting the conventional karyotypic analysis. Based on the literature review, it is presumed that the genes present in the proximal 14q interval are deregulated through the process of heterochromatinization that occurs in the short arm of the chromosome. In this way, it is evidenced that clinical diagnoses, such as facial dysmorphisms observed in the presence of ring chromosomes without apparent loss of chromosome material may be related to changes in chromatin constitution, leading to a change in gene expression due to the positioning. Already the vulnerability to infections and behavioral disorders can be attributed to the 14q32 region. **Conclusion:** The clinical case report presented evidence that even a ring chromosome without associated deletion can lead to serious clinical presentations. It should be remembered that the etiological diagnosis of the deficiencies is essential for genetic counseling, and that the most commonly used CGH Array and NGS sequencing

platforms will have difficulty in identifying structural anomalies without associated losses or gains.

B-319

Novel Biochemical Markers Help Aid in Stratifying Patients at Risk of Preeclampsia and Adverse Events*

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Objective: To develop sensitive and specific objective biochemical markers to help aid in diagnosing preeclampsia.
Relevance: Preeclampsia is a pregnancy complication characterized by high blood pressure, presence of protein in the urine, edema, sudden weight gain, headaches, and changes in vision. Preeclampsia occurs in five to eight percent of all pregnancies. In the United States alone, Preeclampsia is responsible for about eighteen percent of all maternal deaths and fifteen percent of premature births. It is also the leading cause of premature delivery. To date, no objective biochemical marker has been found with high sensitivity and specificity to diagnose preeclampsia accurately. The current strategy to diagnose preeclampsia is through the detection of protein in the urine and onset of high blood pressure during the late second and third trimester pregnancy. However, these symptoms are also present in some normal and many other pregnancy complications such as gestation hypertension, thus increasing the number of false positives. Recent studies on maternal serum protein analysis by proteomics have shown upregulation of placental and hepatic proteins. Two of the upregulated proteins, Pappalysin (PAPP-A, a IGFBP-4 protease and PAPP-A2, a IGFBP-5 protease, produced by placenta) and glycosylated form of fibronectin (preferential binding to SNA and other lectins reflecting sialic acid and fucose carbohydrates) mostly produced by the liver were studied. **Methodology:** Specific monoclonal antibody based ELISAs for GlyFn (AL-160), Pregnancy-Associated Plasma Protein A2 (PAPP-A2, AL-109 C_{cap}-C_{det}, AL-167 C_{cap}-N_{det}), Eosinophil Major Basic Protein (proMBP) (AL-159, $proMBP_{cap}$-$proMBP_{det}$) PAPP-A-proMBP Complex (AL-112, PAPP-A_{cap}-$proMBP_{det}$,) and proMBP-Angiotensinogen (proMBPAGT, AL-111, $proMBP_{cap}$-AGT_{det}) were developed and validated. Preeclampsia status was evaluated using these biomarkers in serum samples from 545 pregnant women (PE, Control, PIH, Undiagnosed) with gestation age 20 to 35 weeks in two subsets of samples. A mathematical algorithm based on 2 decision point using PAPP-A2, GlyFn, protein urea, blood pressure have been evaluated for stratifying the patients the risk of PE and adverse events. **Validation:** ELISAs were very specific to the measured analyte and did not cross-react with other related analytes in the family. ROC analysis for each ELISA was used to calculate the area under the curve (sensitivity and specificity) of diagnosing PE vs Controls. GlyFn and PAPP-A2 ELISAs resulted in AUROC of 1.0 and 0.99 for study 1 and ROC of 0.98 and 0.99 for study 2. PAPP-A-proMBP, proMBP-proMBP and proMBP-AGT had low AUROC of 0.72, 0.64, and 0.52, respectively. Clinical cut-off was established for GlyFn and PAPP-A2 and their serum measurements showed a good concordance with the delivery status (concentrations near the cutoff delivered close to term and elevated concentrations delivered very pre-term). **Conclusions:** GlyFn and PAPP-A2 serum measurements suggest that these proteins play a critical role in preeclampsia and PAPP-A-proMBP, proMBP-proMBP and proMBP-AGT serum levels may not play a significant role in preeclampsia diagnosis. The unique combination of placental (PAPP-A2) and hepatic (GlyFn) protein biomarkers increases the sensitivity and specificity of PE diagnosis over 95%.
*Research Use Only

B-320

Comparison of Blood Lead Level Among School Children in Different Cities of Nepal

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Background: Lead has caused serious public health problems in many parts of the world. Southeast Asia is still suffering from high disease burden from lead poisoning. Children are particularly vulnerable and even relatively low levels of exposure can cause serious health conditions. Establishing the prevalence of Blood Lead Level (BLL) shall help to screen the susceptible children and can prevent them from serious complications with early interventions. **Methods:** The cross sectional study was done on 100 school going students, 50 from industrial city Birgunj and 50 from capital city Kathmandu. Questionnaire was used to collect data. Capillary blood samples were drawn to measure Blood Lead Level. Lead Care II was used to measure Blood Lead Level. Blood Lead Level >5 µg/dl was considered as elevated BLL. SPSS ver. 22 was used to analyze the data. **Results:** The mean BLL in Birgunj came out to be 20.33±9.36 µg/dl. Mean BLL in male was 21.08±8.87µg/dl whereas that for female was 19.46±10.92 µg/dl. All the children in the study from Birgunj have elevated BLL and 84% of them have BLL >10 µg/dl. The mean BLL in children from Kathmandu was 7.01 ± 4.08 µg/dl. Mean BLL in male was 8.08 ± 4.20 µg/dl whereas that for female was 6.35 ± 3.93 µg/dl. About 62% of the children in the study from Kathmandu have elevated BLL and 12% of them have BLL >10 µg/dl. The difference in mean BLL of the children from Kathmandu and Birgunj came out to be statistically significant. ($P < 0.05$) **Conclusion:** The prevalence of BLL in children from industrial city Birgunj is alarmingly high compared to children from Kathmandu. Children exposed with chipped paints, lead acid batteries have comparatively high level of Blood Lead Level. Further study in large population is required to address the current situation regarding the lead exposure to children.

B-321

Rapid decline of fetal lung maturity testing at the University of Minnesota

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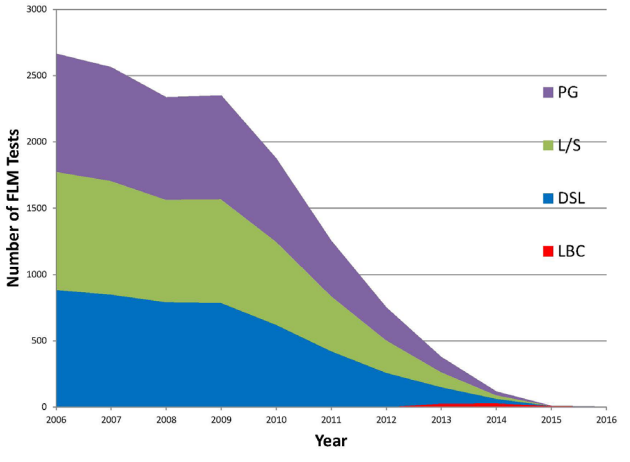
Background: Fetal lung maturity (FLM) testing, first developed in 1971, has been utilized over the years to assess the potential risk for development of respiratory distress syndrome (RDS). The University of Minnesota Medical Center (UMMC) has served as a reference laboratory for FLM testing since 1975, performing thin layer chromatography quantitation of amniotic fluid lecithin:sphingomyelin ratio (L/S), phosphatidylglycerol (PG), and disaturated lecithin (DSL). However in recent years there has been a sharp decline in our FLM testing volumes, leading us to question whether these assays are clinically necessary. **Methods**: Our laboratory information system was queried from 2006 to 2016 for terms associated with FLM testing: desaturated lecithin (DSL), lecithin-sphingomyelin ratio (L/S), phosphatidylglycerol (PG), and lamellar body count (LBC). DSL, L/S, and PG were performed until 2015, and LBC from 2013 to 2016. The lipid assays were done by thin layer chromatography, and the LBC was validated on our hematology platform. Clinicians likely ordered DSL, L/S, and PG for a single patient, but we did not confirm this with chart reviews. **Results:** Graph of test volume plotted by year.
Conclusions: FLM testing has rapidly declined at UMMC, from a volume of 2,665 tests in 2006 to 2 tests in 2016. This precipitous decline is likely due to recent changes in clinical practice guidelines issued by the American College of Obstetrics and Gynecology (ACOG) and the Society for Maternal-Fetal Medicine (SMFM). Both sets of guidelines recommend against using FLM testing to guide management, citing studies which demonstrate that lung maturity does not necessarily reflect maturity of other organ systems, and that decisions to deliver should be more broadly based on multiple maternal and fetal parameters, not just fetal lung status. Given the changing clinical landscape, clinical laboratory directors should meet with obstetrics providers to determine whether FLM testing should be discontinued.



B-322

Transplacental Transfer of Fentanyl Administered During Labor and Delivery

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Background :
Fentanyl is commonly given as an anesthetic during labor and delivery. However, the extent of transplacental transfer and fetal exposure is not well studied. In addition, screening for fentanyl in urine by immunoassay is increasingly common due to the opioid crisis and at our institution fentanyl has been detected in neonatal urine. In this study, we reviewed neonates that had urine fentanyl immunoassays performed and the relationship to maternal fentanyl exposure.
Methods:
All neonates with a urine toxicology panel performed as part of clinical care between January 2017 and December 2017 were included. The urine toxicology panel includes a qualitative screen for fentanyl performed by homogenous enzyme immunoassay (Immunalysis Corporation) with a cutoff of 4 ng/ml. The following variables were obtained: neonatal and maternal urine fentanyl results, time of urine collection(s), APGAR scores at 1 minute and 5 minutes, time and dose of fentanyl administration, time of delivery. A two-tailed, unpaired student's t-test was used to compare means such as APGAR scores and time from fentanyl administration to delivery between neonates with positive versus negative urine fentanyl screens. A Fisher's exact test was used to compare proportions.
Results:
Of the 92 neonatal urine fentanyl screens performed in this study, 25 (27%) were positive for urine fentanyl; 24 (96%) of which could presumably be attributed to fentanyl administration during labor and delivery. Eight of the 24 mothers had a urine fentanyl screen prior to fentanyl administration, all of which were negative, supporting the fact that neonatal results were secondary fentanyl administered during labor and delivery. In the remaining 67 neonates with a negative urine fentanyl screen, 59 (88%) of mothers were given fentanyl. There was no statistical relationship between maternal fentanyl administration and likelihood of positive neonatal fentanyl screen (p=0.44). Neonates with positive urine fentanyl had statistically lower APGAR scores at minute 1 (7 vs 8; p<0.05), but no difference in APGAR scores at minute 5 (8.6 vs 8.5; p>0.5). Neonates with positive urine fentanyl also had higher average time from administered fentanyl to sample collection compared to neonates with negative urine fentanyl (2951 vs 1537 minutes; p=0.05). If the total fentanyl dose was >350 mcg, neonates were significantly more likely to have a positive fentanyl screen (p<0.0001; true positive rate (TPR) of 82%; false positive rate (FPR) of 0%). Additionally, neonates with fentanyl exposure >800 minutes were statistically more likely to have a positive fentanyl screen (p<0.0001; TPR of 80%; FPR of 12%). However, total dose and length of exposure could not predict the result of the fentanyl screen in all neonates, as 16 were positive for urine fentanyl with a dose <350 mcg and length of exposure <800 minutes.
Conclusions:
While dose and length of exposure can predict urine fentanyl results in some neonates, there are a group a neonates with exposure to lower doses and/or shorter duration that were positive for urine fentanyl. More studies are needed to determine if genetic or other maternal characteristics such as weight can predict the extent of fetal exposure.

B-323

Serum Brain-Derived Neurotrophic Factor In Children With Coeliac Disease

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Background: Brain-derived Neurotrophic Factor (BDNF) is a neurotrophin that has a protective role in the nervous system and is involved in neural plasticity. It is abundant in the central nervous system, but is also expressed in the gastrointestinal tract. Recently, BDNF was linked to intestinal inflammation with expression in enteric cells and afferent neuronal pathways in animal models of intestinal inflammation and patients with inflammatory bowel disease (IBD). Although BDNF expression has been studied in several inflammatory conditions, scarce data exist concerning CD. Coeliac disease (CD), characterized by intestinal inflammation, has some co-morbidity with neurologic and mental disorders. The aim of this study was to evaluate circulating BDNF concentrations in patients with CD at diagnosis or on a Gluten Free Diet (GFD) for longer than one year and in healthy controls (HC). **Materials and Methods:** Fifty newly diagnosed patients with CD (aged 8.6±3.7 y, 64.0% females), thirty-nine patients on GFD for longer than one year (aged 10.4±3.4 y, 71.8% females) and 36 HC (aged 8.0±1.7 y, 33.3 % females) were included in the study. Along with anthropometric evaluation and standard blood chemistry, serum BDNF levels were measured by a specific immunoenzymatic assay. **Results:** Serum BDNF levels were significantly higher in newly diagnosed patients with CD than in controls (26,110 ± 8,204 *vs.* 19,630 ± 8,093 pg/ml, respectively, p<0.001). Similarly, BDNF levels were higher in patients on GFD than in controls (28,860 ± 7,992 *vs.* 19,630 ± 8,093 pg/ml, respectively, p<0.001). BDNF levels were significantly higher in patients on GFD than in those at diagnosis (26,110 ± 8,204 *vs.* 28,860 ±7,992 pg/ml, respectively, p=0.02). When patients at diagnosis (all of them had positive serology) were compared to those on GFD with negative serology, a trend for higher BDNF levels was observed for those on GFD, although the difference was not statistically significant (26,301±2,668 vs 30,012± 3,675 pg/ml respectively, p=0.09). No difference in BDNF levels was observed between patients at diagnosis and those on GFD with positive serology either. A correlation analysis within groups, showed that BDNF levels are independent of anti-tTG values (patients at diagnosis: r= 0.147, 0>0.31, patients on GFD: r=0.114, p>0.48). **Conclusions:** In conclusion, according to our findings, BDNF levels were higher in patients with CD than HC, regardless of adherence to the GFD. This finding could suggest a protective role of BDNF against chronic intestinal inflammation or chronic stress from the diet. It seems that BDNF plays an important role in the electrophysiological changes occurring in the CNS of CD patients. Nevertheless, data are still scarce concerning the role of BDNF in CD and further investigation is necessary.

B-324

Development of Amino Acid, L-carnitine and Total Protein Assays in Liquid and Dried Microsamples

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Objective: The objective of this study is to develop a simple, high-throughput microsample screening method for total protein, selected amino acids and L-carnitine that are important in the nutrition of preterm infants. The nutritional requirements of premature infants are controlled by neonatologists who must deliver appropriate amounts of protein, fat and carbohydrate to maximize growth while minimizing toxicity. Enteral nutrition includes Human Breast Milk (HBM), bovine-based infant formulas and high protein supplements. HBM is insufficient to meet the requirements of VLBW or early gestational age infants and must be supplemented with protein. The goal of this study is to be able to correlate preterm infant's nutrition administration with their respective blood metabolite/protein concentrations to help neonatologists make informed decisions. To do so, a separate screening assay is under development. In this study, we report concentration of total protein, metabolites and carnitine in HMB and how they compare to other nutritional preterm infant sources.
Methods and Justification: Standard infant formula, a hydrolyzed infant formula and commercially available cow liquid whole milk, were spotted onto Grade 903 filter paper (75 uL) and dried overnight. Protein was measured using the Pierce™ BCA assay in liquid samples so that the new analysis of dried milk spots (1/16th and 1/8th inch) could be compared. Milk source, punch location, and size were evaluated for total protein measurement with bovine whole milk serving as a control. L-carnitine, acylcarnitines and amino acids were extracted from 3/16th dried milk spots punches and analyzed by MS/MS.
Results and Summary: The protein concentrations of liquid bovine whole milk specimens measured by the BCA assay (33.4 g/L) closely matched the manufacturer-stated concentration of proteins (34 g/L for whole milk). Precision of liquid analysis for whole milk samples was less than less than 4%. For dried milk spots (DMS) using 1/8th or 1/6th in punches, the precision was 10%. Protein concentration increased by 20% from center punch to edge of the spot. Amino acid and acylcarnitine concentrations extracted from DMS where very different from those found in Dried Blood Spots (DPS). Glutamic acid (Glu) was the dominant amino acid in bovine whole milk (283 µmol/L) and for HBM(1400 umol/L). The concentrations of amino acids are not "filtered" plasma. The median concentration of glutamate from preterm infants in DBS is 185 µmol/L. The analysis of protein from was satisfactory in bovine whole milk and achieved the concept of a microsample at 25 µL (liquid specimen) per sample analyzed. This is the first study to examine the analysis of DMS for total protein concentrations using the Pierce kit. For MS/MS analysis, only DMS were utilized. The analysis revealed metabolites that are important for evaluation in nutrition, especially Glutamate which has a purported role in gut metabolism and growth. This method will be useful in the development and implementation of a screening test for protein and metabolites in HBM.

B-325

Reducing discard blood draw volumes from subcutaneously implanted ports (PORT) in patients with End Stage Renal Disease (ESRD)

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Background: To monitor clinical status, dialysis and transplant patients with ESRD frequently require blood draws. To preserve their veins and to avoid frequent intravenous access, these patients, especially young children, require PORT placement. Between blood draws, the PORT is flushed with saline and filled with heparinized saline to prevent blood clotting. To avoid contamination from PORT fluids, a fixed amount of blood is withdrawn and discarded before the blood sample is withdrawn for laboratory analyses. Currently, the recommend discard blood volume is 5 mL which is 5 times the reservoir volume of most PORTs and attached catheters. The volume of discarded blood can be significant, particularly in young patients with ESRD who are already anemic and receive Epogen and iron therapy. This can be a leading cause of iatrogenic anemia. In the present study we evaluated the possibility of reducing the discard blood volume from 5 to 3 mL without compromising laboratory results. **Methods**: After obtaining informed consent, 12 ESRD patients who had PORT placed as part of their clinical care were included in the study. The study period was from February to October 2017. Fifty paired blood samples were drawn from these patients for basic metabolic panel (BMP consisting of sodium, potassium, chloride, bicarbonate, urea, creatinine, calcium and glucose) and complete blood count (CBC consisting of hemoglobin, WBC and platelets) for clinical indications only. The study design included blood wastage of 3 mL and collection of additional 2 mL blood for a total volume of 5 mL. This was followed by collection of additional blood as needed for regular laboratory analyses. Along with regular samples analysis (control), 2 mL aliquots (experimental) were also tested at the same time on the same analyzers. Results for BMP and CBC from control and experimental samples were compared using Bland-Altman analysis. Coefficient of correlation (R^2) by regression analysis were also determined. **Results**: On Bland-Altman analysis, the differences between all except 4 control and experimental paired values were within the preset acceptable variability limits. The R^2 for all analytes ranged between 0.90 for calcium to 0.99 for creatinine, urea and hemoglobin ($p<0.0001$). **Conclusion**: For the tested analytes, the discard blood volume can be reduced from 5 mL to 3 mL. This 40% decrease in the amount of wasted blood can have significant impact on reducing iatrogenic anemia. We plan to extend the study to other analytes.

B-326

Development of an Automated Immunoassay for the Measurement of Pregnancy-associated Plasma Protein A (PAPP-A) on the Siemens ADVIA Centaur XP Immunoassay System

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Background: Pregnancy-associated plasma protein A (PAPP-A) is a placenta-derived glycoprotein. During pregnancy, it is produced by the trophoblast. PAPP-A levels in maternal serum rise with gestational age. The functional significance of PAPP-A is unclear. Some studies suggest that reduced PAPP-A concentrations are associated with chromosomal abnormalities in the fetus.[1] Maternal serum PAPP-A assessment between 11 and 14 weeks of pregnancy is reported to have significant utility in screening for Down syndrome and other chromosomal anomalies. A combination of maternal age-related risk, free β-HCG, and fetal nuchal translucency measurements may substantially increase the efficiency of prenatal screening compared to second-trimester screening. Using this approach, various investigators have reported detection rates for Down syndrome of 85–90% at a 5% false-positive rate.[2] **Method:** A chemiluminescent immunoassay for the detection of PAPP-A has been developed. The ADVIA Centaur® PAPP-A Assay† is intended for in vitro diagnostic use in the quantitative measurement of PAPP-A in human serum using the ADVIA Centaur XP Immunoassay System. PAPP-A is bound to microparticles coated with anti-PAPP-A antibody and is then detected by an acridinium ester (NSP-DMAE)-labeled anti-PAPP-A antibody. Following incubation, wash, and magnetic separation steps, acid and base reagents are added. The resulting chemiluminescence is measured. Assay method comparison to B·R·A·H·M·S PAPP-A KRYPTOR was performed per CLSI EP-09-A3 using 101 patient samples. A precision study was executed over 20 days according to CLSI EP5-A3. Linearity and functional sensitivity studies followed CLSI EP06-A and EP17-A, respectively. Per CLSI EP07-A2, the assay was tested for interference from hemoglobin, bilirubin (conjugated and unconjugated), triglyceride, biotin, cholesterol, immunoglobulin G, protein albumin, rheumatoid factor, and human anti-animal antibodies. The assay was also tested for cross-reactivity with alpha-2-macroglobulin, angiotensinogen, angiotensin 1 and 2, sex-hormone binding globulin, human chorionic gonadotrophin, alpha-fetoprotein, and prolactin per CLSI EP07-A2. **Results:** The reportable range of the assay is up to 10 IU/L without dilution, or up to 100 IU/L with automated 1:10 dilution. Linearity has been demonstrated up to 10 IU/L. Functional sensitivity was observed at 0.01 IU/L. In the precision study, the assay demonstrated within-lab CV of 2.9–4.9%. The method comparison of the assay to the B·R·A·H·M·S PAPP-A KRYPTOR returned a slope of 1.07 and an intercept of 0.05 IU/L by Passing-Bablok regression, and a Pearson coefficient (r) of 0.99. The assay demonstrated no interference and no cross-reactivity with the tested cross reactants. **Discussion and Conclusions:** The feasibility of the automated PAPP-A assay on the Siemens ADVIA Centaur XP System has been assessed and the results show an accurate and precise method for the measurement of PAPP-A in human serum. **Reference:** 1.

Fialova L, et. al. Bratisl Lek Listy. 2002;103(6):194–205. 2. Shiefa S. et. al. Indian J Clin Biochem. 2013;28(1):3–12.

†Under development. Not available for sale, and its future availability cannot be guaranteed.

B-327

CALIPER continuous reference curves for biochemical markers: Advantages over traditional partitioned reference intervals

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Background: Despite the critical importance of reference intervals for accurate interpretation of laboratory test results, they have traditionally been severely lacking in the pediatric population. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has made significant strides to close this gap by establishing a pediatric reference interval database based on data from thousands of healthy children and adolescents (www.caliperproject.ca). CALIPER reference intervals have traditionally been partitioned by age, using the Harris & Boyd method to determine statistically significant age partitions. However, analyte concentration does not change abruptly with age, but rather changes dynamically. In this study, we establish continuous reference intervals for biochemical markers using the CALIPER database to provide a more accurate estimate of age-related changes in biomarker concentration. **Methods:** Data from CALIPER subjects aged 1-<19 years were used to establish continuous reference intervals for eight analytes, including alanine aminotransferase, albumin, alkaline phosphatase, total bilirubin, calcium, creatinine, phosphate, and uric acid. Data from subjects <1 year of age were excluded. Continuous reference intervals (i.e. 2.5th and 97.5th quantiles) were established using non-parametric quantile regression via a univariate B-spline with a penalty to impose monotonicity and quantile non-crossing constraints using R software. This method is robust to various departures from assumptions, including normality, symmetry, linearity, and variance homogeneity, as well as outliers. **Results:** Reference curves were established for several biochemical markers, showing the dynamic age-related trends in analyte concentration. A table of reference values for each 6-month age bin was also established. Calcium and alanine aminotransferase concentration remained relatively stable throughout the age range, showing little dependence on age. Total bilirubin, creatinine, and uric acid continuously increased with age. Alkaline phosphatase showed a non-linear relationship with age, increasing until puberty, and subsequently decreasing into adulthood. Although less pronounced, phosphate exhibited a similar age-related dynamic to alkaline phosphatase. **Conclusion:** Continuous reference intervals better reflect the dynamic age-related trend in analyte concentration. However, the feasibility of implementing continuous reference intervals into clinical practice remains an issue, particularly considering the limitations of current laboratory information systems. We provide tables of 6-month age bins to increase their feasibility, although this inherently reduces the accuracy of continuous reference intervals.

Wednesday, August 1, 2018

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

Point-of-Care Testing

B-328

Point of care measurement of creatinine and eGFR in the emergency department

A. Ivanov, T. Tammert-Müürsepp, K. Nõmm. *Tartu University Hospitals, Tartu, Estonia*

Background: Incidence of chronic kidney disease (CKD) around the world is reported to be between 8,5-15,6%. Identifying patients with CKD prior to a diagnostic radiological imaging with nephrotoxic contrast agents will reduce a risk of contrast-induced acute kidney injury (CI-AKI). **Aim:** To compare POC-creatinine and eGFR with central laboratory and assess the impact of POC-creatinine and eGFR testing with immediate feedback to the clinician on the risk of developing CI-AKI. **Methods:** The study was performed during three month period using samples obtained from the Emergency Department of Tartu University Hospital. Creatinine was measured prior to the diagnostic radiological imaging with LIS-connected handheld POC-creatinine meter (Stat Sensor, Nova Biomedical, MA,USA). eGFR was automatically calculated by LIS after connecting the Stat Sensor Creatinine meter to the docking station. At the same time venous blood was collected and tested on Cobas 6000 analyzer (Roche Diagnostics, Switzerland) utilizing a creatinine enzymatic method. The IDMS-traceable abbreviated Modification of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate and report eGFR . **Results:** We have compared the results of 214 patients for creatinine measurement ranged from 16 µmol/L to 610 µmol/L (eGFR from 6 to 207 ml/min/1.73m^2). We used three partitions of eGFR results according to stages 3-5 of chronic kidney disease (<30, 30-60, >60) and evaluated the statistical parameters for each interval. The linear regression analysis demonstrated a slope of 0,79 for creatinine and 0,88/0,72/0,87 for /eGFR<30/ eGFR 30-60/ eGFR >60. POC-creatinine had the mean bias 0,02 µmol/L or 1,6% for creatinine and 0,57/1,33/-2,88 ml/min/1.73m^2 or 5,3/1,7/-4,0% for eGFR compared with laboratory method. The results demonstrated that POC and laboratory methods had no significant bias for creatinine (*p*-0,989), for eGFR <30 (*p*-0,687) and for eGFR 30-60 (*p*-0,313). The significant bias was for eGFR >60 (*p*-0,002). The 90th percentile of laboratory turnaround time for creatinine/eGFR is 69 min, the time to result of Stat Sensor Creatinine meter is 30 sec. **Conclusion:** The data demonstrates that Stat Sensor Creatinine meter is an effective tool for rapid assessment and identification of CI-AKI risk of CKD patients, improve the general workflow while preserving the patient`s quality of life.

B-329

From Paper to Plastic: Re-innovating Ultra Low-cost Electricity-Free Point-of-Care Blood Centrifuge for Resource Challenged Clinical Chemistry Laboratory

A. Gautam, B. Subedi, A. Kumar, N. Koirala. *Dr. Koirala Research Institute for Biotechnology and Biodiversity, Kathmandu, Nepal*

Background

We are witnessing a great paradigm shift from central laboratory-based diagnosis to point-of-care based diagnosis. Currently used centrifuges are heavy, large, expensive and impractical for field clinics, which may have no electricity access. A hand-powered, ultra low-cost, portable centrifuge has long been required in a setting where modern standard centrifuges are impractical. The design and fabrication of hand-powered centrifuge is based on principle of an ancient whirligig string toy operated by spinning. We evaluated the electricity-free centrifugation potential of the hand-powered centrifuge using human blood at point-of-care level.

Methods

We designed and fabricated plastic/paper centrifuge of 12-cm diameter size. It was composed of two circular 1-mm thick plastic/paper discs used for stationery purpose which was bound by an adhesive tapes with two small holes in the center. Two sample capillary holders were glued horizontally to the discs. An extra-strong 62-cm long fishing line passing through centre of discs was used for spinning. Phlebotomy was performed aseptically during remote field clinics and 2-3 ml blood was transferred to small tube. Then it was placed inside the sample holder and rotated by hand for 3-4 minutes. This resulted in a good separation of plasma from the cellular blood components.

Results

We found that hand-powered centrifuge can adequately separate the plasma from anti-coagulated blood within 2-3 minutes. The overall cost and weight of centrifuge is $ 0.5 USD and about 25-30g respectively, which is more practical and cost-effective than modern electric centrifuges. Although the volume of blood used was less, we were able to show good qualitative agreement in terms of centrifugation and separation of pure plasma from anti-coagulated blood between the hand-powered centrifuge and conventional electric centrifuges.

Conclusion

Re-purposing of a simple toy has resulted in a novel point-of-care electricity-free blood centrifuging device for remote clinical laboratory. This device can act as promising alternative to electric centrifuges for point-of-care field diagnosis. Additionally the device and methodology provides a practical alternative when the serum or plasma is required for point-of-care field testing or analysis by diagnostic kits and device (e.g.testing for a number of diseased conditions).

B-330

GEM Premier 5000 Method Comparison Study for Native Capillary Samples

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Background: Capillary blood sampling is increasingly common in medicine and provides several advantages over venous blood sampling: it is less invasive, it requires smaller amounts of blood volume and it can be performed quickly and easily in Point of Care settings. However, if carried out incorrectly, capillary blood sampling can cause inaccurate test results, pain and tissue damage. In addition, the small volumes involved and the variability in sample quality based on puncture site and technique make capillary sampling particularly susceptible to errors during the pre-analytical phase. **Methods:** This method comparison study using samples collected via capillary puncture was performed at one external Point of Care (POC) setting in combination with one internal laboratory setting, located at IL, which simulates a POC setting by use of POC operators. A minimum of 120 native capillary samples were collected per analyte. Collection of capillary samples was performed according to established guidelines (pre-warming of the puncture site for increased blood flow, removal of first drop to avoid tissue fluid contamination, no milking to prevent hemolysis, removal of air gaps within the sample and mixing for sample homogeneity). At the internal site, additional contrived samples were included to span the reportable range for each analyte. **Results:** Good correlation between GEM Premier 5000 and GEM Premier 4000 was observed for all analytes tested. All slopes were between 0.9 and 1.1 and r > 0.950 (see table 1). In addition, bias at the medical decision levels (MDL's) was within the total allowable error (TEa) for all analytes tested. **Conclusion:** The data illustrates excellent analytical performance with a clinical sample type that is known to have challenging pre-analytical characteristics. Based on the results obtained in this study, native capillary performance on the GEM Premier 5000 is substantially equivalent to the GEM Premier 4000.

Table 1: Method Comparison results for GEM Premier 5000 vs. GEM Premier 4000

Analyte	Slope	Intercept	r
pH	0.935	0.494	0.975
pO_2 (mmHg)	1.008	2.545	0.996
pCO_2 (mmHg)	1.000	1.000	0.980
Na^+(mmol/L)	1.015	-1.750	0.981
K^+(mmol/L)	1.000	0.100	0.995
Ca^{++}(mmol/L)	1.050	-0.016	0.998
Cl^-(mmol/L)	1.000	-1.000	0.995
Glu(mg/dL)	0.966	4.775	0.997
Lac(mmol/L)	1.000	0.000	0.995
Hct(%)	1.003	-0.407	0.987
tHb(g/dL)	1.028	-0.470	0.994
O_2Hb(%)	1.000	0.802	0.997
COHb(%)	0.988	-0.269	0.999
MetHb(%)	1.000	-0.100	0.998

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Comparison of the time required for manual and semi-automated Urinalysis and Pregnancy testing with associated EMR manual entry errors.

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Background: Urinalysis (UA) is commonly used for the evaluation of proteinuria, glucose, urinary tract infection, and pregnancy (hCG/UPT) in Ob/Gyn patients. In our Ob/Gyn clinics we perform >50,000 UA tests/year (>94% being performed manually). The same clinics manually perform >30,000 UPT/year. Studies have indicated that this subjective evaluation is only moderately accurate compared with the evaluation of urine using automated instrumentation. In addition to the result being subjective it also requires significant time to perform and document results in the patient's chart (EMR). With the availability of semi-automated POCT instruments (in our case Siemens Clinitek Status Connect) capable of performing and transmitting to the EMR both UA and UPT results it is possible to reduce preanalytic (bar code reader), analytic (instrument resulting), and postanalytic (transmission to the EMR) errors. The objective of this study is to determine the potential time savings associated with using the Clinitek Status instrument-read solutions for routine urinalysis dipstick and UPT vs. manual testing and to determine the transcription error rate for manual entry of results in the EMR. **Methods:** Data was collected prospectively in 5 Ob/Gyn clinics, 2 of which currently have Clinitek Status Connect instruments that are not connected to the EMR. The IRB approved study specifics are: 1) Conduct a time study in Ob/Gyn clinics comparing workflow of UA and UPT associated with manual read vs. Clinitek Status. Total test time includes the time required to document the results in the EMR (test time + EMR entry time). 2) Review the results from UA and UPT results manually entered in the EMR to identify transcription error rates associated with manual entry. Total tests resulted were used to calculate error rate (i.e. Chem 7=7 tests) **Results:** The difference of test time and total test time to perform for a Chem 6-10 UA (N=67) was significantly less for the Clinitek Status (0.77 min.; $p<0.001$ and 0.64 min; $p<0.002$, respectively) while the difference in test time for the Chem 2 (N=30) manual read was significantly less (0.09 min; $p=0.005$) but not for the total test time (0.08; $p=0.33$). For the UPT the Clinitek Status has a 5 min test time to report a negative result thus the test time was significantly greater (1.45 min; $p=<0.001$), however, the total test time was the same (0.61 min; $p=0.059$).Preliminary results for the clinics studied found a transcription error rate of 0.3-1.7% for individual UA results (N=3550 individual test results). No transcription errors were seen for UPT. Although unanticipated but perhaps not unexpected, we also found that ~8% the UA results and ~12% UPT results were not documented in our EMR. **Conclusion:** As anticipated the Clinitek Status UA system was more efficient than the manual process. Manual UPT testing was faster than the Clinitek Status although the total test time was the same. Once connected to the EMR the Clinitek Status will eliminate the transcription errors and lack of documentation of some test results. Partial study funding was received from Siemens.

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Precision and Total Error of the Afinion™ HbA1c Dx Test* with Fingerstick Samples

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Background: The objective of this study was to estimate the between-instrument and between-operator components of precision for the investigational point-of-care (POC) Afinion HbA1c Dx test using fingerstick whole blood samples in a moderate complexity laboratory setting. This analysis, taken together with previous results, estimated total precision for fingerstick samples and total error for the Afinion HbA1c Dx test. **Methods:** Following a study design recommended by the Food and Drug Administration (FDA), 60 subjects were enrolled across three clinical sites and four levels of %HbA1c. Three test operators each collected two fingerstick samples from each subject and tested one sample on each of two analyzers. There were a total of 90 fingerstick measurements at each %HbA1c level. The within-run, between-operator, and between-instrument components of variance were calculated for each level using ANOVA. Total precision was calculated from the between-instrument and between-operator variance from the present study together with the within-run (including between-lot), between-run, and between-day components from two previous studies. The resulting total coefficient of variation (CV), together with bias estimates from one of the prior studies was used to estimate total error. **Results:**** The fingerstick precision components calculated from this study are shown in Table 1. Across the four %HbA1c levels the between-instrument imprecision was 0.00-0.47% CV, the between-operator imprecision 0.00-0.26% CV, and within-run imprecision 1.09-1.39% CV. The total precision was 0.882.00% and the total error ranged from 2.86-4.68%.

Table 1: Summary of Fingerstick Precision Study Results

HbA1c Level	Grand Mean %HbA1c	N (M)	Between Instrument		Between Operator		Within Run	
			SD	CV(%)	SD	CV(%)	SD	CV(%)
Low	5.33	15 (90)	0.0076	0.14	0.0000	0.00	0.0742	1.39
Threshold	6.51	15 (90)	0.0192	0.30	0.0000	0.00	0.0811	1.25
Medium	8.41	15 (90)	0.0000	0.00	0.0218	0.26	0.0919	1.09
High	12.20	15 (90)	0.0570	0.47	0.0000	0.00	0.1389	1.14

Notes: N (M) is the number of subjects (number of test results). CV is SD from ANOVA divided by Grand Mean.

Conclusions: The Afinion HbA1c Dx test is precise across its measurement range when using fingerstick whole blood samples. The total error estimates are well below the current NGSP requirements of ±6% total allowable error across the assay range. *The Afinion HbA1c Dx test is not FDA cleared for sale in the U.S. **Under FDA review for pre-market notification

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Giant Magnetoresistive Based Handheld System for Rapid Detection of Human NT-proBNP

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Background:
Since the significant discovery of cardiac natriuretic peptide hormones, a great deal of research has identified 2 peptides derived from pro-B-type natriuretic peptide (proBNP), namely BNP and N-terminal-proBNP (NT-proBNP), as valuable plasma biomarkers for indication of heart failure (HF) and other cardiac diseases. Many *in vitro* diagnostic kits of BNP/NT-proBNP for assessing HF risk have been successfully commercialized. Biochip-based assay for biomarkers detection using giant magnetoresistive (GMR) sensors and magnetic nanoparticles (MNPs) have been developed by different research groups. However, no portable and handheld GMR biosensor system has been reported yet. In this study, a novel handheld GMR detection system with integrated microfluidics was used to detect human NT-proBNP, which revealed advantages of high sensitivity and specificity, and real-time signal readout. The developed assays have great potential for the final development of simple, rapid, automatic and cost-effective point-of-care testing (POCT). **Methods:** The immunoassay process is set up based on sandwich-type format. NT-proBNP capture antibodies (Abs) were printed and immobilized on different sensors on one GMR chip with functional surface. Integrated with microfluidic system, the chip was assembled with plastic substrate and valves to form a test cartridge. After the cartridge was connected with the handheld detection analyzer, TBST buffer (Tris-buffered saline, 0.05% Tween 20) was pumped onto sensor surfaces to wash off unbound Abs. Then sample prepared by diluting NT-proBNP analytes to desired concentrations in assay buffer was loaded into sample entry well which was prefilled with biotin labeled NT-proBNP detection Abs. Capture Ab-analyte-detection Ab (biotin) sandwich complex was formed on sensor surface as sample solution flowed along microfluidic channel. At last streptavidin labeled MNPs (SA-MNPs) were introduced and bound onto sensor surfaces via the interaction between SA and biotin. Binding of SA-MNPs to sensor surface can be real-time recorded by the handheld analyzer. Higher detection signal reflected more MNPs binding on sensor surface. **Results:** *In vitro* detection of human NT-proBNP using a new handheld GMR biosensor platform was well established. The assay can be completed within 20 min, which is much shorter than conventional and widely used enzyme-linked immunosorbent assays (ELISA). The novel assay provides analytical ranges of 15-20000 pg/mL for NT-proBNP, and its detection limits is around 10 pg/mL. NT-proBNP with varied concentrations were spiked into human plasma, and recoveries of 85-115% are observed. It is also shown that the assay is not interfered with hemoglobin, fibrinogen, human anti-mouse antibody and rheumatoid factor. **Conclusion:** The developed technology platform for GMR based immunoassay can

sensitively and specifically detect human NT-proBNP. Not only the assay time has been shortened, but also simple and automatic assay operation has been accomplished. Hence, we believe it can be further integrated and developed for POCT diagnostics.

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High sensitivity cTnI capability demonstrated on the Minicare Point of Care Platform

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Background: Cardiac Troponin (cTn) has been accepted as the biomarker of choice for detection of acute myocardial injury (AMI). Advances in assay technology have led to high sensitive (HS) cTn assays that have a profound impact on clinical practice, supporting clinical decision making based on results at presentation and 1 hour after admission. Next to the time between measurements, workflow plays an important role in the turn-around time. Currently, HS-cTn tests are only available on central lab-systems and the associated logistics to get a sample to the lab and the result reported back to the physician significantly impacts the time to a disposition decision. Point-of-care (POC) assays have the potential to drastically shorten this turn-around time, especially when combined with a first measurement in an ambulance setting. This enables more rapid decision making. Here we evaluate an improved version of the current Philips Minicare cTnI POC test under development, which has the potential to combine the benefits of HS cTnI protocols with a POC workflow. **Objective:** Evaluate the capability of the Minicare HS-cTnI test under development to meet the criterion[1] for HS of having a 10% CV < 99th percentile. **Methods:** The evaluation is based on the Clinical Laboratory Standards Institute (CLSI) guidelines. Li-heparin whole blood and Li-heparin plasma samples were used to establish Limit of Quantitation (LoQ) and to perform a method comparison study between Minicare and Abbott Architect high-sensitivity troponin I (n=426). **Results:** With an assay time of less than 10 minutes, the 10% CV LoQ was established at <10 ng/L. The method comparison between Minicare and Abbott Architect high-sensitivity troponin I resulted in a Pearson correlation coefficient of 0.92. The Passing-Bablok regression demonstrated a slope of 1.59, so expressed in Architect units, the 10% CV LoQ on Minicare would be <7 ng/L. For the Abbott Architect high-sensitivity troponin I assay the 99th percentile has been established at 34 ng/L (male) and 16 ng/L (female). This would lead to a 10% CV LoQ for the Minicare HS-cTnI assay well below the 99th percentile. **Conclusions:** With demonstrated HS cTnI capability on the Minicare platform, we show the potential to support a 0/1 h sampling protocol, with the speed of a POC workflow. This enables rapid and safe rule-out of patients with suspected AMI in the ED.

[1] *F. S. Apple and P. O. Collinson, "Analytical Characteristics of High-Sensitivity Cardiac Troponin Assays" Clin Chem. 2012 Jan; 58(1):54-61.*

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Comparative evaluation of urine dipsticks with regard to urinary leukocyte screening

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Background:
Urinary tract infections (UTI) are responsible for over 8.1 million office visits per year.[1] Screening with urine dipsticks is a quick and cost-effective method for initial patient evaluation that can prevent unnecessary testing. One screening method involves testing for leukocyte esterase, the product of leukocyte presence in infection. The objective of this study is to compare the Siemens Multistix® 10SG reagent strips/CLINITEK Status®+ Urine Chemistry Analyzer, CLARITY CLA-URS10 reagent strips/UROCHECK 120 urine analyzer, YD DIAGNOSTICS URISCAN 10 SGL Strips/OPTIMA urine analyzer, and TECO DIAGNOSTICS URS-10 strips in their ability to detect leukocyte esterase with contrived solutions and confirming findings in native specimens.
Methods:
This study consists of two parts. The first consists of testing urine strips in duplicate with contrived samples containing known quantities of leukocyte esterase. The second assessment involves visual and instrument testing of 62 clinical urine specimens spanning the reporting range of the urine leukocyte dipsticks, in an attempt to confirm the findings of the contrived study.
Results:
The Siemens and CLARITY tests matched the expected results of the contrived leukocyte esterase solutions without issue. The YD DIAGNOSTICS and TECO DIAGNOSTICS tests exhibited negative bias with the moderate/2+ solution. Analysis of clinical sample results showed overall visual assessment agreement versus the Multistix®10 SG Reagent Strips at 85.4% with the CLARITY strips, 48.3% with the YD DIAGNOSTICS strips, and 24.1% with the TECO DIAGNOSTICS strips. Compared to the CLINITEK Status®+ Urine Chemistry Analyzer, the CLARITY device exhibited 57.8% overall agreement and the YD OPTIMA device exhibited 12.9% overall agreement.
Conclusion:
Clinical outcomes are highly dependent on the results of initial diagnostic screening in the Point of Care environment, as they determine the preemptive course of action or confirmatory tests required for expeditious treatment. This study demonstrates that significant negative bias exists when comparing several urine tests versus the Multistix®10 SG Reagent Strips, and that this bias will translate to the point of care environment.
Footnotes
[1] https://www.nichd.nih.gov/health/topics/urinary/conditioninfo/affected

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Performance evaluation of the point of care cardiac troponin T assay

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Background: The cobas h 232 POC system (Roche Diagnostics) is a point-of-care testing device for troponin T assay. Herein, we aim to evaluate the analytical performance of the CARDIAC POC Troponin T assay (Roche Diagnostics) on the cobas h 232 POC system.
Methods: The repeatability and within-laboratory imprecision of the CARDIAC POC Troponin T assay were evaluated by using the Roche CARDIAC POC Troponin T 2-Level control according to the CLSI documents EP15-A3. Since the concentration of troponin T of Level 1 control solution was less than the lower limit of quantification (e.g., 40 ng/L) on the cobas h 232 POC system, only Level 2 control solution was used. In addition, repeatability was determined by running n=10 replicates per patient sample. Linearity of the CARDIAC POC Troponin T assay was determined using five levels of patient samples according to CLSI document EP6-A. The method comparison between Elecsys Troponin T high sensitive (TnT-hs) assay on the cobas e411 analyzer and CARDIAC POC Troponin T assay on the cobas h232 POC system was performed based on the CLSI document EP9-A3.
Results: The repeatability (%CV) and within-laboratory imprecision (%CV) of Level 2 control solution (mean troponin T, 441.6 ng/L) was 8.5% and 8.6%, respectively. The repeatability of patient samples was 7.5% at 88.7 ng/L and 7.2% at 454.6 ng/L. Linear range of the CARDIAC POC Troponin T assay was confirmed between 54.0 ng/L and 1347.7 ng/L. Compared with the high sensitive troponin T assay, the linear correlation equation (correlation coefficient) was y=0.985x-20.8 (r = 0.988).
Conclusion: Our data suggest that the CARDIAC POC Troponin T assay could be useful in cases where the POC troponin T testing is required.

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Polynomial Regression Analysis Techniques to Evaluate Variables. A Practical Example with Internal Proficiency Data Comparing AccuChek® Inform II with cobas® and i-STAT® methods.

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Background: In the Sentara Hospitals system the performance of AccuChek® Inform II glucose meters is monitored with both cobas® and i-STAT® methods. We report the results of the analysis of data obtained in a four year continuous internal proficiency testing program, with polynomial multivariate regression analysis techniques. **Methods**: AccuChek Inform II (Roche Diagnostics), i-STAT cartridges (Chem8 and CG8+, Abbott Diagnostics), cobas c501, c311 (Roche Diagnostics). Patient specimens, with values in the interval 50 - 600 mg/dL, obtained by venipuncture were assayed in parallel and within 30 minutes with AccuChek Inform II and either i-STAT or the laboratory method in thirteen facilities. The data were collected and transferred electronically to Minitab® (version 17, Minitab Inc.) statistical software and analyzed with regression analysis statistical techniques. **Results**: Since the three glucose methods display an increase in variance for increasing values of glucose the orthogonal regression model (y=3.7 +0.96 x) was compared with the weighted least squares model (y=4.7+0.95x). Clearly the two models showed very similar estimates of the regression parameters which would not affect either quality assurance or clinical applications. The weighted polynomial regression model was used to compare location, year, and reference method. The ANOVA table showed that there were no statistically significant differences between the regression lines for facility (P=0.68), year (P=0.96) and reference method (P=0.22). Stepwise regression (x to enter=0.15, x to remove=0.25), identified

the reference method (Adjusted MSE=0.8, Adjusted MS for reference methods=3.3, F=4.01, P=0.46) as the single major contributor to the unexplained variation. Similar results were obtained with forward selection (x to enter=0.25, Adjusted MSE=0.8, Adjusted MS for reference method=3.3, F=4.01, P=0.046) and backward elimination (x to remove=0.25, Adjusted MSE=0.8, Adjusted MS for reference method=3.3, F=4.01, P=0.046). The pure error test did not show statistically significant lack of fit (F=0.8, P=0.7) and this was corroborated by the lowess of the plot of the standardized deleted residuals by the glucose value. Furthermore, the plot of the relative and the absolute bias of the glucose value, as determined with the AccuChek Inform II method, versus the value, as determined with the reference methods, was within the CLIA's criterion (target value +/- 6 mg/dL, or +/- 10%, greater). **Conclusions**: The weighted polynomial regression analysis showed similar, stable performance in thirteen facilities for four years. The relative and absolute differences between the glucose values as obtained with AccuChek Inform II versus paired values as obtained with the reference methods were within the CLIA's criterion. The statistically but not clinically significant differences between regression lines for the reference methods may be due to either design and/or calibration. However, since the data were generated by an unplanned consecutive QA operation and not from a planned experimental design, the error may not have been random, but may have been due to the effect of one or several latent variables and this bias may have induced imprecision in estimating the regression parameters. Finally, both the electronic transfer of data and the use of a statistical software, such as Minitab, were of the paramount importance for conducting these studies.

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Method comparison and bias estimation at clinical decision levels for creatinine and urea measurements with ABL90 Flex Plus blood gas analyzer and Dimension Vista

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Background: Creatinine and urea are relevant parameters to monitor renal function. The ABL90 Flex Plus blood gas analyzer has recently incorporated a new electrode-based biosensor cassette capable of measuring these parameters with the same sample volume and measuring time. The availability of these parameters as POCT in clinical settings such as emergency department or critical care units could be very useful in order to make clinical decisions faster and reducing waiting times. The aim of this study was to estimate bias at clinical decision levels in order to establish if creatinine and urea measurements are interchangeable between the ABL90 Flex Plus and a central laboratory method (Dimension Vista). **Material and methods:** ABL90 Flex Plus (Radiometer®) and Dimension Vista (Siemens Healthineers®), as a comparative method, were used for the study. According to Clinical and Laboratory Standards Institute (CLSI) protocol EP09-A2-IR, 40 whole blood heparinized samples were analysed by duplicate. Linear regression and comparability at clinical decision levels between both analyzers were calculated. The allowable bias was established according to desirable Total Error (dTE) based on biological variation criteria. Statistical analysis was performed with Analyse-it® software.

Medical decision levels (mg/dL)	Estimated bias (%)	95% CI	Allowable difference (%)
CREATININE			
0.3	-2.6	-12.3 to 9.5	±8.9
0.6	-5.6	-8.8 to -0.7	±8.9
1.2	-7.2	-8.9 to -4.5	±8.9
6.0	-8.4	-10.5 to -4.7	±8.9
UREA			
13	-12.9	-23.9 to -3.6	±15.6
56	7.5	4.8 to 9.6	±15.6
107	10.4	6.6 to 13.5	±15.6
200	11.9	7.5 to 15.8	±15.6

Results:

Conclusions: The estimated bias was lower than the allowable bias at different clinical decision levels. Therefore, creatinine and urea patient results are interchangeable between ABL90 Flex Plus and Dimension Vista. This ensures no impact on patient care when using alternatively both analyzers.

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comparision of two point of care gasometers: gem premier 4000 (werfem group) and epoc (alere)

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Background: Point of care tests (POCT) are defined as "bedside assistance and wherever the patient is, or where the decisions are made by the health team, wherever they are". Given the importance of the parameters evaluated in the gasometry, it is important that the response time is reduced to the maximum, and therefore, gasometers of the POCT type are a very helpful tool. The objetive was to evaluate the correlation and transferability of the results between GEM PREMIER 4000 (WERFEN GROUP) and EPOC (ALERE) gas meters for the parameters measured relative to the acid-base balance and oxygenation and ion state. **Methods:** Prospective study, in which 66 gasometry samples were included: 24 corresponded to patients hospitalized in the Intensive Care Unit and 42 to patients from the extraction area of the laboratory of our hospital during a period of 4 months (January to April 2017). The samples were processed in parallel, 60 venous gasometries and 6 arterial blood gases, and to minimize the preanalytical error, the analysis of the samples was performed in the 2 teams following a sequential order: first in the GEM PREMIER 4000 analyzer (Werfen Group) and immediately after in EPOC (ALERE). The results were evaluated by the Pearson correlation coefficient and the bilateral significance level. **Results:**

Parameters	Pearson correlation (CI 95%)	Bilateral significance level
pH	0.986 (0.965-0.993)	0,000
Partial pressure of CO2	0.984 (0.993-0.993)	0,000
Partial pressure of O2	0.995 (0.979-0.998)	0,000
Ion sodium	0.887 (0.393-0.960)	0,000
Ion potassium	0.989 (0.975-0.994)	0,000
Ion chloride	0.945 (0.909-0.967)	0,000
Ionic calcium	0.824 (0.712-0.892)	0.000
Glucose	0.984 (0.947-0.993)	0,000
Lactate	0.985 (0.973-0.991)	0,000
Hemoglobin	0.916 (0.824-0.953)	0,000
Oxygen saturation	0.959 (0.893-0.980)	0,000
Total CO2	0.982 (0.967-0.989)	0,000
Bicarbonate	0.984 (0.974-0.990)	0,000
Hematocrit	0.956 (0.923-0.975)	0,000

Conclusion:

- Both teams, GEM PREMIER 4000 (WERFEN GROUP) and EPOC (ALERE), present very significant correlations ($p < 0.0005$) for all the parameters studied. - Therefore, both gasometers are transferable methods and therefore, the results can be interchangeable. - Given that the ALERE EPOC team is a small equipment that can be easily transferred by the medical team, it has many advantages such as: - Handling and transport of the minimum sample. - Determination in the place of assistance of the patient. - Reduction of time for decision making. - Rapid stratification of patients.

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Assessment of Strip Lot and Meter Variation with the Nova Statstrip® Lactate Point of Care Device

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Background: Lactate is a metabolic by-product of tissue hypoxia and is a marker of metabolic stress in the body. It holds prognostic value in many conditions including sepsis, heart failure and respiratory failure. Reproducible measurement of lactate is critical for clinical outcome in patient care. No consensus reference method for the measurement of lactate currently exists and as such assessment of method accuracy is problematic. **Objective:** To evaluate the analytical performance of the Nova Statstrip® lactate meters and strips. **Methods:** Precision was assessed using Nova QC materials as well as venous and arterial whole blood specimens. Variation in strip lot (n=3) was evaluated using one lactate meter and Nova Biomedical linearity material. Patient correlations (n=50) were conducted using three different lot number of strips

and one lactate meter compared with the Radiometer ABL837 lactate method. Meter to meter variation was assessed using venous cord blood specimens (n=40) and one strip lot number compared with the Radiometer ABL837 lactate method. **Results:** Imprecision (CV) was dependent upon both specimen type and lactate concentration. CV was greater with arterial specimens (11-13%) than venous specimens (4.8-6.1%) or Nova QC materials (1.9-8.3%). CV also increased with decreasing lactate concentration. The following regression equations describe the relationship between: a) Individual lactate strip lots and the assigned lactate linearity material concentration $y = 0.974x + 0.161$ (strip 1) $R^2 = 0.998$; $y = 0.999x + 0.122$ (strip 2) $R^2 = 0.998$; $y = 0.989x + 0.055$ (strip 3) $R^2 = 0.998$ b) Patient correlations and individual lactate strip lots $y = 0.791x - 0.145$ (strip 1) $R^2 = 0.975$; $y = 0.796x - 0.158$ (strip 2) $R^2 = 0.973$; $y = 0.819x - 0.187$ (strip 3) $R^2 = 0.984$ c) Venous cord blood correlations and individual lactate meters $y = 0.876x - 0.114$ (meter 1) $R^2 = 0.941$; $y = 0.925x - 0.330$ (meter 2) $R^2 = 0.932$; $y = 0.859x + 0.122$ (meter 3) $R^2 = 0.920$ **Conclusions:** Strip lot and meter variation detected with the Nova Statstrip® lactate meter were clinically insignificant. Specimen type was found to influence method CV.

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Hyperglycemia from Eating Preserved Sweet Plums?

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Background:
On 27th May 2017, a Point-of-care testing (POCT) glucose result of 33.1 mmol/L was observed in a ward diabetic patient. The test was repeated with a result of 9.3 mmol/L. On 28th May 2017, a POCT glucose result of 33.1 mmol/L was observed in the same patient. The test was repeated with a result of 9.8 mmol/L. We suspected the elevated POCT glucose results could be due to pre-analytical factors. On both occasions, we found out that the patient was eating preserved sweet plums 1-2 hours before the blood test. We designed a study to replicate the clinical scenario to validate our suspicion of possible finger contamination with food containing sugar resulting in a false high glucose.
Methods:
A non-diabetic subject was recruited for the study. POCT glucose was performed for the subject after handling preserved sweet plum, followed by no cleaning with alcohol swab, cleaning the fingertip with alcohol swab once and swabbing 3 times. POCT glucose was analyzed using Roche Accuchek Inform II glucometer.
Results:
POCT glucose results before and after handling preserved sweet plums (with no cleaning with alcohol swab) were 5.2 and 19.1 mmol/L respectively. POCT glucose was 8.4 mmol/L after cleaning fingertip with alcohol swab once. When the fingertip was cleaned thoroughly with 3 times swabbing with alcohol swab, the POCT glucose was 5.2 mmol/L.
Conclusion:
Inadequate cleaning of fingertip with alcohol swab prior to POCT glucose testing in patients who handled food containing sugar could produce falsely elevated blood glucose result.

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Principles and Practice of Point-of-care Environmental Stress Testing: Static and Dynamic Robustness of a WBC-Differential Instrument and its Role in Detecting Highly Infectious Diseases

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Background: Point-of-care (POC) instruments that perform rapid diagnoses at or near patient sites must withstand environmental stresses (ES). Changes in white blood cell and differential (WBC-DIFF) counts determined by optical scanning can help identify communicable infections in epidemics of highly infectious diseases like Ebola. This project enables rapid and accurate detection of critical infections to help prevent outbreaks. Our goals were to evaluate the environmental robustness of a POC hematology instrument (HemoCue, Sweden) that determines WBC-DIFF in capillary whole blood and to establish the principles and practice of this new POC field. **Methods:** We investigated the simultaneous environmental robustness of the instrument and its reagents. Whole-blood capillary samples were obtained from consented human volunteers (IRB 294372-10). The instrument (FDA investigational in the US) reports total white blood cell count (WBC) and a five-part differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). For dynamic temperature, humidity was held constant to eliminate that confounding variable, while for humidity perturbations, temperature was held within the manufactures acceptable range. Whole-blood measurements were performed in hot and cold environments using Tenny ES chambers to establish dynamic low and high temperatures from 18-30 ºC (manufacturer's specifications) versus room temperature control. ES chambers simulated high and low static and dynamic humidity conditions less than and greater than 90% RH, the manufacture specified upper limit. The results were compiled and significance was determined using Student's t-test for paired differences. ANOVA was performed on serial clusters of paired differences. **Results:** Month-long storage of reagents at static high temperatures (~50°C) and static and dynamic humidity (25% to <90% RH) did not affect paired differences significantly ($P>0.05$). However, results indicate that dynamic high temperature (> 30°C) impairs WBC-DIFF measurements. In addition, humidity exceeding manufacturer specifications (>90% RH) affects WBC-DIFF system (instrument and reagents) reliability, accuracy, and performance. We optimized an ES module comprising written instructions, protocols, and specifications for national POCT policy and guidelines, which will be particularly useful in limited-resource settings and countries faced with temperature, humidity, dust, and other physical challenges. **Conclusions:** Our research protocol uniquely evaluated *both* instruments and reagents simultaneously during temperature and humidity stress, in order to consistently and realistically simulate conditions encountered in community hospitals, primary care sites, patient homes, and field settings during crises, such as disasters and pandemics. We conclude that this WBC-Diff instrument and future POC devices: a) must be evaluated for environmental limits, b) can perform well within objectively defined temperature and humidity brackets, and c) should be designed to withstand ES in limited-resource settings. We used these results and well-defined protocols [see Louie RF et al., *Global Point of Care*, AACC Press-Elsevier, 2015, pp. 293-306] to establish a unique principles and practice module for environmental stress testing of instruments and reagents. Robustness is needed to improve diagnosis and evidence-based decision making in regions of risk at points of need, including screening in primary contact sites. Additionally, national point-of-care policy and guidelines with the ES module will help assure the quality of diagnostic performance and enhance standards of care worldwide.

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Presepsin and N-terminal pro-B-type Natriuretic Peptide Improve Outcome Prediction of Patients admitted with Sepsis to the Emergency Department

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Background: Assessment of disease severity of sepsis at the time of initial presentation is crucial as the mortality of severe sepsis or septic shock is 30 to 60% whereas the mortality of sepsis without organ failure remains below 10%. It has been reported that implementation of early goal directed therapy into the treatment of severe sepsis and septic shock in the emergency department (ED) may reduce mortality. This underlines the necessity to adequately identify patients with a high risk of poor outcome in the ED as early as possible. The point-of-care (POC) test PATHFAST Presepsin has been shown to provide early prognostication in sepsis. N-terminal pro-B-type natriuretic peptide (NT-proBNP) may provide information about cardiovascular organ failure which occurs commonly in severe sepsis and septic shock. **Objectives:** We thought to evaluate presepsin (PSEP) in combination NT-proBNP to assess disease severity and outcome prediction in patients with sepsis directly after admission to the ED. **Methods:** PSEP and NT-proBNP concentrations were measured in plasma samples which were drawn from 106 patients with sepsis according to sepsis-1 definition at the time of admission to the ED. PSEP and NT-proBNP were determined using PATHFAST Presepsin (LSI Medience corporation, Tokyo) and Elecsys NT-proBNP (Roche Diagnostics). PCT, CRP and creatinine were measured using routine clinical chemistry methods in the central laboratory. Primary endpoint was death within 30 daysThe combined endpoint "major adverse events" (MAE) consisted of at least either the primary or at least one of the secondary endpoints intensive care, mechanical ventilation or dialysis. **Results:** 15 patients died and 26 patients exhibited MAEs during 30 day follow up. The number of non-survivors were 3 (4.4%) and 13 (34.2%) in patients with sepsis (n=68) and severe sepsis or septic shock (n=38), respectively. MAEs occurred in 6 (8.8%) and 20 (52.6%) patients with sepsis and severe sepsis or septic shock. Median values of NT-proBNP and PSEP in Sepsis were 193 and 693 ng/L compared to 555 and 1407 ng/L ($p<0.0001$) in severe sepsis or septic shock. ROC analysis revealed AUC values of NT-proBNP and PSEP of 0.714 / 0.715 and 0.707 /0.737 for risk prediction of MAE and death, respectively. The logistic regression of simultaneous assessment of NT-proBNP and PSEP revealed elevated AUC values of 0.736 and 0.745 for risk prediction of MAE and death, respectively.

Conclusion: NT-proBNP and PSEP demonstrated strong relationship with disease severity and outcome in patient with sepsis admitted to the ED. The simultaneous assessment of NT-proBNP and PSEP provided higher risk prediction of MAE and death than both markers alone. The PATHFAST POC system allows early determination of PSEP and NT-proBNP in parallel from whole blood within 17 min directly in the ED and may improve the management of sepsis.

B-344

Automatic Mixing as a Patient Blood Management Tool

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Objective:
The aim of the studies were to show how automatic mixing of an arterial blood gas (ABG) syringe (*safe*PICO, Radiometer Medical) ensures comparability of hemoglobin results obtained on the ABL90 FLEX blood gas analyzer at the Point of Care, with a laboratory hematology analyzer (XN-series, Sysmex). Patient blood management is a focus area with the purpose to follow the highest standards in blood conservation. This also includes collection of the lowest possible blood volume with the highest possible sample quality.
Methodology:
Heparinized venous blood was split into two ABG syringes; A). A 1 mL ABG syringe with no automatic mixing capacity B). A 1.5 mL ABG syringe containing a mixing ball for automatic mixing The two syringes were initially mixed and stored equally, and the syringes were handled by one laboratory person only. An EDTA sample was simultaneously drawn and measured on the Sysmex.
Results:
Data was collected at five US sites and pooled. A regression analysis and bias plots were performed comparing hemoglobin measured in each of the two syringes measured on the blood gas analyzer to the hematology analyzer.

Syringe	n	Slope	Intercept (g/dL)	Correlation coefficient r^2	Mean difference to Sysmex (g/dL)	Confidence interval (g/dL)
A	99	0.79	3.6	0.515	-0.89	-0.5 to -1.3
B	99	1.02	-0.1	0.986	-0.22	-0.2 to -0.3

Conclusion:
Using syringe B with automatic mixing at the Point of care results in a significant better correlation of hemoglobin with the laboratory analyzer. Automatic mixing ensures a homogenous sample and accurate hemoglobin results. Accurate hemoglobin results are essential also when the focus is on patient blood management, i.e. collecting the lowest possible blood volume with the highest possible sample quality in the conservation of patient blood.

B-345

Performance evaluation of the Point of Care Minicare BNP assay

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Background: B-type natriuretic peptide (BNP) is increased under conditions of myocardial pressure or volume overload, primarily in patients with heart failure. It is therefore considered to be a useful marker of myocardial function. International guidelines[1] recommend its use for the exclusion of heart failure in patients with dyspnea. Point-of-care (POC) systems for BNP can replace laboratory systems to support the routine evaluation of patients presenting to an emergency department (ED) with acute dyspnea and may accelerate the throughput and disposition of at-risk patients in the ED. The Minicare BNP test (currently under development) is a rapid POC in-vitro diagnostic test for the measurement of BNP in low volumes (30 µl) of human EDTA whole blood, EDTA plasma and capillary blood.
Objective: To assess the performance characteristics of the Minicare BNP assay under development.
Methods: Analytical performance of the Minicare BNP assay was evaluated following applicable CLSI guidelines. Studies were performed at the Department of Internal Medicine III — Cardiology and Angiology (Innsbruck), Diagnostiek Voor U (Eindhoven), Future Diagnostics Solutions (Wijchen) and at Philips (Eindhoven) using human whole blood, plasma and capillary blood specimens.
Results: Limit of Blank (LoB) and Limit of Detection (LoD) were determined in EDTA plasma to be 3.3 ng/L, and 5.8 ng/L respectively. The Limit of Quantitation ($LOQ_{20\%}$) at 20% CV was <10 ng/L for both EDTA whole blood and EDTA plasma. Total imprecision was found to be between 6.7% and 9.7% at BNP testing concentrations of 93 - 3984 ng/L. The sample type comparison study was done on capillary whole blood, venous EDTA whole blood and EDTA plasma samples (n≥150) and resulted in a Pearson correlation coefficient (r) of 0.98-0.99 and a Passing-Bablok slope between 1.03 and 1.09. The normal value study on 158 healthy subjects showed a median BNP concentration of 16 ng/L. 99% of values (158/159 for whole blood and 156/158 for plasma) were below the generally recommended cut-off of 100 ng/L for the exclusion of acute heart failure, with no significant influence of sample type. Method comparison studies against a core laboratory assay (Siemens ADVIA Centaur BNP), demonstrated a Passing-Bablok slope of 1.06-1.08 and a Pearson correlation coefficient (r) of 0.92-0.93 (n=187). The percentage of agreement using the cut-off value of 100 ng/L was 92%.
Conclusion: The Minicare BNP assay is a fast and easy-to-use test which is intended for the near-patient setting. The test requires only a droplet of blood that can be obtained by capillary draw or from venipuncture. The Minicare BNP assay under development is a robust and accurate assay as demonstrated by its high analytical sensitivity, low imprecision and high correlation to an established core lab assay. Capillary blood samples can be used on Minicare BNP and deliver results which are highly comparable to venous blood measurements.
[1] *Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012, Eur. Heart J. 33 (2012) 1787-1847.*

B-346

Evaluation of "Rapidchip® fFN," a new rapid quantitative measurement of fetal fibronectin to predict preterm labor

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Background: Testing for fetal fibronectin (fFN) is used to predict preterm labor in pregnant women between 22 weeks and 35 weeks of gestation. A rapid and quantitative point-of-care (POC) test kit for fFN ("Rapidchip™ fFN; Sekisui Medical Co., Japan) was recently developed that is based on the principle of lateral flow immunochromatography. We evaluated the analytical performance of a system consisting of this new Rapidchip® fFN assay and a RapidPia® POC testing instrument (Sekisui Medical Co., Japan).
Methods: The immunochromatography test strip in the cassette housing contains two monoclonal antibodies that react with fFN. Colloidal gold-labeled antibody coats the conjugate pad and the other antibody coats the detection zone of the membrane. The labeled antibody forms a complex with fFN that reacts with the antibody coating the membrane to form a red line at the detection zone. The intensity of the red line depends on the concentration of fFN. This assay system measures the signal intensity and converts it to quantitative and qualitative values based on a cut-off of 50 ng/mL. All patients underwent serial vaginal sampling for fFN measurement from 22 to 32 weeks of gestation by collection of cervicovaginal secretions during speculum examination. We compared the performance of this fFN assay with that of ELISA by using 81 samples of cervicovaginal secretions.
Results: The lower limit of detection of the assay was 25 ng/mL, while the upper limit of quantitation was 500 ng/mL. No prozone effect was observed in samples with fFN concentrations from 259 to 6475 ng/mL. At fFN concentrations of 75 ng/mL, 175 ng/mL, and 352 ng/mL, the within-run C.V. (n = 5) was 6.2%, 3.5%, and 2.2%, respectively. Compared to ELISA, the sensitivity of the fFN assay was 91.7% (11/12) and its specificity was 97.1%(67/69), with an overall agreement rate of 96.3% (78/81). The performance of this new fFN assay system was equivalent to that of ELISA, and only 3 out of 81 samples showed discrepant results compared with the gold standard assay.
Conclusion: This new fFN assay based on immunochromatography provided results in only 10 minutes, allowing rapid diagnosis and management of patients with a high risk of preterm labor. The results of this study suggest that the fFN assay system is not only a useful diagnostic tool for inpatient perinatal care, but also for reducing the risk of preterm labor and predicting premature delivery in the outpatient setting.

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Evaluation of the precision performance on the GEM® Premier™ 5000 at CHR Citadelle (Belgium)

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The GEM Premier 5000 is a new critical care analyzer for providing rapid analysis of whole blood samples at the point of care or in a central laboratory. This analyzer contains a single, all-in-one PAK to provide quantitative measurements of pH, pCO_2, pO_2, sodium, potassium, chloride, ionized calcium, glucose, lactate, hematocrit, total bilirubin and CO-Oximetry parameters. These measurements aid in the diagnosis of a patient's acid/base status, electrolyte and metabolite balance and oxygen delivery capacity. The system precision performance of the GEM Premier 5000 was evaluated at the CHR Citadelle Hospital compared against three Critical Care analyzers from different manufacturers: the GEM Premier 4000 (Instrumentation Laboratory), ABL™ 90 (Radiometer) and the RapidPoint® 405 (Siemens). The evaluation was conducted by clinical personnel at CHR Citadelle Hospital. Three (3) levels of external, traditional-ampule based quality control (QC) material was used in the evaluation (RNA Medical QC). QC material was run for six (6) days, two (2) runs per day and three (3) replicates per run (36 samples). First samples were run as soon as the system was available after cartridge installation to evaluate the performance of the system at this critical time. Total SD was calculated for each tested analyte. Results were compared against a selected criteria derived by CLIA recommendations. Results are summarized in table 1. Most of the precision results were within the recommended criteria. However, pCO2 for RapidPoint 405 and Glucose and Lactate for the ABL 90 showed results outside the criteria. The source of the observed imprecision and possible implications in analytical performance will be discussed in this poster. Conclusion: The results obtained during the verification process demonstrate that the GEM Premier 5000 system offers optimal performance for point-of-care or laboratory testing with consistent performance from the start of the cartridge uselife.

Table 1: Precision performance summary

Analyte and Level	GEM Premier 5000		GEM Premier 4000		Radiometer ABL 90		Siemens RapidPoint 405		Performance criteria
	Mean	SD (or CV%)	Mean	SD (or CV%)	Mean	SD (or CV%)	Mean	SD (or CV%)	
pH (Leve 1)	7.117	0.006	7.128	0.008	7.145	0.004	7.097	0.017	0.02
pH (Level 2)	7.433	0.004	7.422	0.009	7.412	0.004	7.392	0.007	0.02
pH (Level 3)	7.674	0.006	7.650	0.014	7.602	0.003	7.589	0.010	0.02
pCO_2 mmHg (Leve 1)	71.47	2.91%	70.09	2.70%	66.25	1.53%	85.95	6.42%	4.0%
pCO_2 mmHg (Level 2)	43.69	1.06	41.97	0.82	40.61	0.66	46.6	2.5	2.5
pCO_2 mmHg (Level 3)	22.09	0.53	21.28	0.81	21.66	0.3	22.46	1.05	2.5
Glucose mg/dL (Leve 1)	81.09	1.78%	74.34	2.18%	70.28	8.79%	76.68	1.42%	5%
Glucose mg/dL (Level 2)	201.84	1.57%	189.09	3.55%	183.94	5.40%	196.62	1.36%	5%
Glucose mg/dL (Level 3)	304.03	2.22%	280.16	4.31%	273.81	4.32%	288.41	1.24%	5%
Lactate mmol/L (Level 1)	0.60	0.02	0.63	0.05	0.55	0.11	Results not provided		0.2
Lactate mmol/L (Level 2)	2.45	4.02%	2.39	3.51%	2.38	10.47%			7.5%
Lactate mmol/L (Level 3)	7.05	4.67	6.56	4.52	6.62	5.53%			7.5%

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Evaluation of Whole Blood Basic Metabolic Panel Assay with ED Samples

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Background: A Basic Metabolic Panel (BMP) is one of the most commonly ordered blood tests that provides Emergency Department (ED) physicians with a quick assessment of a patient's electrolyte and fluid balance, blood glucose level and kidney function. A whole blood (WB) BMP cartridge based on electrochemical creatinine, blood urea nitrogen (BUN), and total CO_2 (tCO_2) assays for the GEM Premier analyzer (Instrumentation Laboratory) is currently in development. This is an addition to the electrolytes and metabolites currently offered on the GEM Premier analyzers. The goal of this clinical evaluation is to compare the WB analytical performance of the GEM Premier BMP cartridge in a point-of-care (POC) setting to the established reference methods for ED samples. **Methods:** Random heparinized WB samples were obtained from the ED at HCMC and evaluated by POC staffs. The WB samples were analyzed on the GEM Premier analyzer (IL) with four BMP cartridges over the course of eight weeks. As reference methods, the WB samples were then assayed on a standard GEM Premier 4000 analyzer (IL) for Na+, K+, Ca++, Cl-, glucose, lactate, pH, pCO_2 and hematocrit. The plasma portions were assayed on the Cobas 6000 analyzer (Roche Diagnostics) for creatinine, BUN, and tCO_2. Some of the native samples (<10% per analyte) were adjusted to expand the measured ranges. **Results:** The WB creatinine, BUN and tCO_2 results from GEM Premier BMP cartridges correlated well with those obtained from plasma samples on the Cobas analyzer across the ranges of the tested samples, the same was true for the correlation of rest of the analytes to GEM Premier 4000 (results summarized in Table 1). **Conclusion:** Strong correlations were observed between the GEM Premier BMP and the reference methods. GEM Premier BMP cartridge can provide reliable results with quick turnaround time in POC environments like the ED.

Table 1. Method Correlation Results for the GEM Premier WB Assays *vs.* References [(a)] Roche Cobas 6000 or [(b)] GEM Premier 4000 by [(c)] Passing-Bablok or [(d)] Deming Regression Analysis

Analyte	Slope	Intercept	r	N	Sample Range	MDL1 (Bias)	MDL2 (Bias)	MDL3 (Bias)
BUN [(a,c)]	1.04	0.85	0.999	114	3.5 - 107.5 mg/dL	6.0 (1.09)	26.0 (7.3%)	50.0 (5.7%)
Crea [(a,c)]	1.05	0.034	0.999	115	0.375 - 13.63 mg/dL	0.6 (0.06)	1.6 (0.11)	6.0 (5.2%)
tCO_2 [(a,c)]	0.90	1.87	0.983	111	6.4 - 42.3 mmol/L	6.0 (1.28)	20.0 (-0.5%)	33.0 (-4.2%)
Na^+ [(b,d)]	1.01	-1.89	0.987	127	109 - 169 mmol/L	115 (-0.3)	135 (-0.1)	150 (0.1)
K^+ [(b,c)]	1.00	0.00	0.999	132	0.6 - 16.2 mmol/L	3.0 (0.0)	5.8 (0.0)	7.5 (0.0%)
Cl^- [(b,c)]	1.00	-2.00	0.996	129	40 - 147 mmol/L	90 (-2.2%)	112 (-1.8%)	n/a
Ca^{++} [(b,c)]	1.00	0.01	0.999	128	0.21 - 4.47 mmol/L	0.37 (0.01)	0.82 (0.01)	1.58 (0.6%)
Hct [(b,d)]	1.04	-0.55	0.995	123	15 - 65 %	21 (0.3)	33 (0.8)	55 (1.7)
Glu [(b,c)]	1.05	-6.01	1.000	126	5 - 679 mg/dL	45 (-3.8)	90 (-1.7%)	180 (1.7%)
Lac [(b,c)]	1.00	0.00	1.000	131	0.4 - 20.0 mmol/L	2.0(0.0)	5.0 (0.0%)	n/a
pH [(b,d)]	0.99	0.11	0.992	120	6.81 - 7.53	7.30 (0.003)	7.35 (0.002)	7.45 (0.001)
pCO_2 [(b,c)]	1.00	0.00	0.990	120	21 - 65 mmHg	35 (0.0)	50 (0.0)	70 (0.0%)

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Evaluation of the performance of albumin-to-creatinine ratio with automated urinalysis

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Background: Taiwan has the highest end-stage renal disease prevalence among all countries and the costs on maintenance dialysis imposes a great financial burden on National Health Insurance. As a marker of kidney damage, albumin creatinine ratio (ACR) is recommended by National kidney Foundation (NKF) for detecting early stage of chronic kidney disease. Routine urinalysis provide an opportunity for early detection microalbuminuria. Herein, we evaluated the accuracy of semi-quantitative chemical methods from SIEMENS NOVUS PRO12 dipstick for ACR. **Methods:** We collected 1029 random urine samples underwent urinary analytic tests from outpatient department. Urinary protein, microalbumin and creatinine were measured by SIEMENS Novus with PRO12 dipsticks, a semi-quantitative test. The urinary ACR was also calculated. The reference method was turbidimetric immunoassay (Autokit Micro Albumin kit, WAKO) performed by HITACHI LST008, an automatic quantitative assay. Within 2 hours, the accuracy of PRO12 dipsticks for ACR was assessed by percentage of agreement with the value measured by quantitative assay, and the sensitivity, specificity, positive and negative predictive values for microalbuminuria were calculated. **Results:** The percentage of exact agreement in ACR was 81.9% between PRO12 dipsticks and quantitative assay. The percentage of agreement within one level between two methods was 98.5%. When ACR > 30 mg/g defined as positive results, the sensitivity, specificity, positive and negative predictive values for microalbuminuria were 87.2%, 91.6%, 91.5%, and 87.3%, respectively. Among 1029 cases, there were 778 cases with negative results of urinary protein analyzed by conventional dipsticks. However, 149 of 778 (19.2%) cases were positive for ACR measured by PRO12 dipsticks. Moreover, 111 out of 149 (74.5%) cases were confirmed positive ACR by quantitative assay. **Conclusion:** Early-stage of kidney disease is usually asymptomatic which requires routine urinalysis for detection. Urinary ACR measured by SIEMENS Novus with PRO12 dipsticks was shown to be a reliable test for screening of microalbuminuria.

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Molecular Influenza Testing on the cobas Liat PCR System in Different Clinical Settings

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Background: The cobas Liat PCR System (Roche, Indianapolis, IN) performs molecular Influenza A/B testing on Universal Transport Media (UTM) inoculated with nasopharyngeal swabs. Results are available in 20 minutes, comparable to many non-molecular rapid Influenza tests. Thomas Jefferson University Hospital placed Liats at the Point-of-Care (POC) in three different clinical settings during two consecutive flu seasons to evaluate providers' courses of action and determine if an available 20 minute molecular flu test would alter treatment decisions. **Methods:** Liats were placed in TJUH Center City Emergency Department (ED), Women's Primary Care (WPC), and 700 Walnut Urgent Care Center (UCC). Nasopharyngeal swabs were ordered, collected on suspected flu patients, and used to inoculate UTM. 100uL UTM was removed and tested on the Liat. The remainder was tested by methods normally utilized by each site: ED and WPC samples were sent to TJUH Microbiology Laboratory for testing on the GeneXpert (Cepheid, Sunnyvale, CA); UCC performs rapid flu tests using the Sofia Influenza A+B Fluorescent Immunossay (FIA) (Quidel Corporation, San Diego, CA). Samples were blinded for testing and matched at the end of the study. Providers were instructed not to use Liat results. Data was collected by questionnaire, including symptoms, immunization status, diagnosis, treatment, and whether or not treatment would have changed with an available 20 minute molecular result. **Results:** Survey responses varied greatly (see table below). UCC providers responded unanimously that treatment would not have changed. Providers would have changed treatment for approximately 50% of ED and WPC patients if 20 minute molecular results were available. Decision variables included 1) no isolation 2) earlier disposition (discharge/admission/other), 3) earlier prescription of Tamiflu. Eight discrepant results were observed. **Conclusion:** Clinical use of POC molecular flu testing varies from provider to provider. Standardized guidelines would be useful in driving decision-making, perhaps a subject for future studies.

Location	If results had been available in 20 min, would treatment have been different? Yes	No	Did not follow protocol - no PT ID, nothing sent to lab, no questionnaire	No answer	Discrepant results	Practice change: more detailed responses
Urgent Care Center *n*=75	0	65	10	0	6 discrepant results Liat: Flu A Pos / Sofia: Flu A/B Neg / # 2 Liat: Flu B Pos / Sofia: Flu A/B Neg / # 2 Liat: Flu A/B Neg / Sofia: Flu A Pos / # 1 Liat: Flu A/B Neg / Sofia: Flu B Pos / # 1	• No provider would have changed his or her treatment
Women's Primary *n*=30	10	11	8	1	No discrepant results	• Eight patients would have been given Tamiflu earlier
ED *n*=120	49	45	1	25	2 discrepant results Liat: Flu B Pos / Sofia: Flu A/B Neg / # 2	• 9 patients would have been dispositioned earlier • 3 patients would not have been placed in isolation • Seven patients would have been given Tamiflu earlier

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Performance evaluation of AQT90 FLEX procalcitonin assay

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Background: Early diagnosis and differential diagnosis of sepsis using appropriate blood markers is very important for lowering the mortality rate and reducing the unnecessary use of antimicrobial agents through appropriate antibiotic treatment in sepsis patients. The AQT90 FLEX procalcitonin (PCT) assay (Radiometer, Australia) is easy-to-use and point-of-care testing analyzer that can be used in an emergency room or an intensive care unit where rapid diagnosis and differential diagnosis are required. The aim of this study was to assess the analytical performance of this new AQT90 FLEX PCT assay and to compare it with the previously used Cobas e602 Elecsys BRAHMS PCT (Roche, Switzerland). In addition, we assessed the correlation of PCT results among different specimen types. Methods: We evaluated the analytical performance of AQT90 FLEX PCT including precision, linearity and correlation with the Cobas e602 Elecsys BRAHMS PCT in accordance with CLSI EP 15-A, EP 6-A, and EP 9-A2. Additionally, the PCT levels in EDTA whole blood, EDTA plasma, and serum samples obtained from the same individual were compared to evaluate the matrix influence. Results: The AQT90 FLEX PCT assay showed good linearity (linearity range, 0.17-88.8 ng/mL, $R^2 > 0.99$). The within-run and total coefficients of variations were within 5% for low and high level quality control materials (3.0% and 4.7%, 1.7% and 1.8%, respectively). The carryover rate was 0.03%. In the methodology study using EDTA plasma sample, the Pearson's correlation coefficient (r) was 0.999 (95% CI, 0.998 to 0.999), but the PCT value on AQT90 FLEX PCT was about 22% higher than that on the cobas e602 Elecsys BRAHMS PCT, on average. In the correlation study between EDTA whole blood and plasma on AQT90 FLEX analyzer, Pearson's correlation coefficient (r) was 0.999 (95% CI, 0.999 to 1.000). The PCT levels in EDTA whole blood were, on average, about 6% higher than those in EDTA plasma. Conclusion: The AQT90 FLEX PCT assay showed suitable analytical performance with respect to precision, linearity and carryover rate. The PCT value in EDTA whole blood showed higher than that in EDTA plasma, on average. In conclusion, the AQT90 FLEX PCT assay provided reliable and precise PCT results, and can be used to diagnose sepsis rapidly not only as point of care testing but also in clinical laboratory, through application of appropriate cut-off level according to different sample types.

B-352

Accuracy and Reproducibility Evaluation of ADAMS HA-8180V using HbA1c Quality Targets Model (QTM)

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Background: HbA1c, a key parameter in diabetes management, is now being recommended for diagnosis and screening. Hence, the laboratory quality management should focus on both accuracy and reproducibility of the chosen HbA1c device. To aid with this, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a model that is based on the concept of total error which includes both bias (related to accuracy) and imprecision (related to reproducibility). The model grades the performance in five categories: fail, pass, pass in bronze, pass in silver, and pass in gold [Weykamp C, Little RJ, Sacks DB

et al, Clin Chem 61:5, 752-759 (2015)]. The ARKRAY ADAMS A1c HA-8180V was recently cleared by FDA. The device measures HbA1c (IFCC mmol/mol and NGSP%) in human whole blood and hemolysate samples using ion exchange high performance liquid chromatography (HPLC). The purpose of this study was to evaluate the accuracy and reproducibility of ADAMS HA-8180V using the HbA1c QTM. **Methods:** A precision study was performed per CLSI EP05-A3 *Evaluation of Precision Performance of Quantitative Measurement Methods*. EDTA whole blood samples from four donors at HbA1c concentrations of ~5.0%, ~6.5%, ~8.0%, and ~12.0% were utilized in the study. Whole blood and hemolysate samples were run in duplicate in two runs per instrument per day for 20 days using three HA-8180V analyzers. **Results**: This pilot study showed that the ADAMS A1c HA-8180V system results for all samples fell in the "pass in gold" (Fig. 1) range of the HbA1c QTM plot. **Conclusion:** The ADAMS A1c HA-8180V system is a robust, safe, and accurate method for routine HbA1c measurement in laboratories. Further studies evaluating its performance across different laboratories in the US is required.

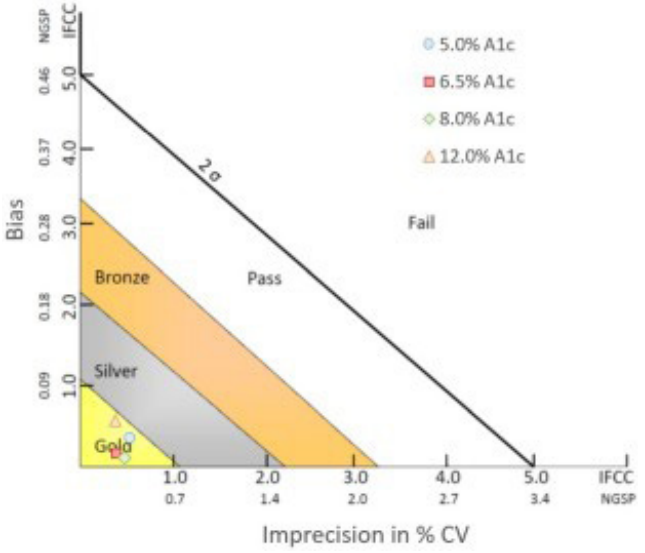

Figure 1: Bias vs. Imprecision of the HA-8180V performance on the QTM at the measured patient values.

B-353

Evaluation of the cobas Liat group A streptococcus assay in the Express Care setting

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Background: The cobas Liat (Roche) is an FDA-approved and CLIA-waived point-of-care (POC) polymerase chain reaction (PCR) system for detecting group A streptococcus (GAS) from throat swabs, generating results within approximately 20 minutes. A prior study conducted at Mayo Clinic using experienced laboratory technologists and strict contamination prevention protocols showed Liat results were accurate compared to routine real-time PCR results. Here, we examine the performance of the Liat system in the hands of end users in a POC environment (i.e., staff in Mayo Clinic Express Care (MCEC) retail clinics).Methods: Patients (age 3-65 years) presenting for clinical evaluation for GAS at two MCEC locations were recruited. For each patient, two throat swabs were collected. One specimen was sent to the Clinical Microbiology laboratory for routine real-time PCR testing on the LightCycler platform (Roche), with results available within 4-6 hours. The second was analyzed at the POC by MCEC staff using the cobas Liat system. POC results were compared to central laboratory results for concordance. The crossing point (C_p) of the routine laboratory test was recorded for discrepant results. In cases of Liat assay failures, the failure was noted on the study log and the specimen was retested until results were obtained. Weekly environmental swabs of the Liat instrument and surrounding work area were collected at both locations over 13 weeks and tested for GAS environmental/amplicon contamination by the routine real-time PCR and the Liat assay.Results: A total of 468 patients were enrolled. Concordance between Liat and the routine PCR was 97.2% (455/468). Sensitivity and specificity of the Liat GAS assay were 97.6% (206/211) and 96.9% (249/257), respectively. Routine real-time PCR results were positive and Liat results were negative in 8 samples. However, 7 of those discrepant results were associated with a low positive (C_p greater than 30) by the routine PCR assay. No environmental contamination was detected by either the Liat or the routine real-time PCR tests. Assay failures were observed in 6.6% (33/501) of Liat runs.Conclusions: The Liat PCR system provides accurate GAS results in the clinic setting with no evidence of environmental contamination. The reduced result turnaround time compared to routine real-time PCR (~20 minutes vs. 4-6 hours) would allow for more rapid patient management decisions. Therefore, the Liat should be considered an option for POC GAS testing.

B-354

A novel tool to relate glucose meter performance to clinical outcome: The Insulin Dose Error Assessment (IDEA) Grid

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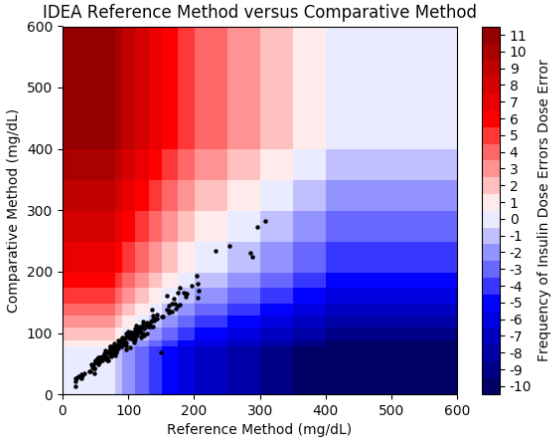
Background: The Clarke grid, Parkes error grids and Surveillance error grid were developed from expert opinion to assess the clinical accuracy of glucose meters. In the past decade there have been technological advances in the analytical performance of glucose meters and numerous insulin-dose protocols developed for local hospitalized and community patients. To relate accuracy of glucose tests and clinical use of insulin, an error grid could express glucose error in units of the 'size of error of insulin dose' administered, customized for the local insulin protocol for a specific patient group. **Objective:** To develop a grid to display the relationship between glucose error and the associated error in insulin dose, using an individual institutional insulin protocol. **Methods:** The effect of 0.5 mg/dL differences between reference and test methods on the risk of insulin dosing error was simulated using a published insulin dosing protocol (Karon et al., 2010). Data are displayed on a grid of reference glucose and meter glucose values with increasing color intensity applied as the size of clinical error in units of insulin dose errors increases. To evaluate a glucose meter, paired glucose data for the reference and test methods are plotted on the error grid and a histogram represents the frequency of insulin dose errors. **Results:**

Figure 1. IDEA error grid analysis: Patient correlation data (n= 199) measured by reference and test glucose methods are plotted on the error grid. A frequency histogram of the insulin dose errors of the patient results depict 94.5% of insulin doses were within +/- 1 dose, 99.5% within +/- 2 doses. **Conclusions:** The IDEA grid is a useful tool that describes differences in glucose measurement in terms of insulin dosing error. This grid is capable of being individualized to an insulin dosing protocol to enable objective assessment of clinical risk attributed to analytic glucose meter error.



B-355

Evaluation of health outcomes after the implementation of rotational thromboelastometry in patients undergoing cardiac surgery.

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Background: Viscoelastic tests (rotational thromboelastometry, ROTEM®), together with the implementation of a specific algorithm for coagulation management in cardiac surgery, enable perioperative coagulopathy to be better controlled. **Methods:** Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass. The incidence of allogeneic blood transfusions and clinical postoperative complications were analyzed before and after ROTEM® implementation. **Results:** Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, p=0.026) during the perioperative period. In the group monitored with ROTEM®, decreased incidence of transfusion was observed for packed red blood cells (31.3% vs 19.8%, p=0.002), fresh frozen plasma (9.8% vs 3.8%, p=0.008), prothrombin complex concentrate administration (0.9% vs 0.3%, p=0.599) and activated recombinant factor VII (0.3% vs 0.0%, p=0.603). Increased incidence was observed for platelet transfusion (4.8% vs 6.8%, p=0,530) and fibrinogen concentrate (0.9% vs 3.5%, p=0.066), tranexamic acid (0.0% vs 0.6%, p=0.370) and protamine administration (0.6% vs 0.9%, p=0.908). Similar results were observed in the postoperative period, but with a decreased incidence of platelet transfusion (4.8% vs 3.8%, p=0.813). In addition, statistically significant reductions were detected in the incidence of postoperative bleeding (9.5% vs 5.3%, p=0.037), surgical reexploration (6.0% vs 2.9%, p=0.035), and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, p=0.026). **Conclusion:** The monitoring of hemostasis by ROTEM® in cardiac surgery, was associated with decreased incidence of allogeneic blood transfusion, clinical hematologic postoperative complications and lengths of ICU stay.

B-356

Evaluation of Point of Care Process Efficiency in an Emergency Department Using Abbott i-STAT

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Background: Utilization of cartridge-based point of care tests (POCT) is growing. Rapid turnaround times (TAT) are especially important in the emergency department (ED) care setting. Cartridge errors and unusable results in testing can result in increased staff workload, increased reagent consumption, delayed diagnosis and adverse patient outcomes. To understand clinical and economic impact of these errors, we evaluated the frequency of occurrence of POCT errors in an ED dataset as well as estimated how these errors impact TAT. **Methods:** A retrospective analysis was conducted using de-identified records for 15,479 i-STAT® cartridges run in the ED at Oregon Health & Science University between December 2015 and August 2016. Data were collected from the device middleware and EHR for three cartridge types: blood gases (CG4+), chemistry (Chem8+) and Troponin. The frequency of cartridge errors and unusable results (indicated in the data as a ***, <> or <or> code) and the effect of variables on error frequency such as operator ID and time/date of testing were evaluated for the three cartridge types. We also investigated the effect of device errors on TAT, calculated as the elapsed time from the first POCT order to the return of the first valid result from either a repeated POCT or core laboratory test. A second dataset was used to estimate Chem8+ and Troponin cartridge waste based on the difference between the number of cartridges used for patient testing (tests ordered) versus the number of reported results over a two-year period. **Results:** A total of 935 cartridge errors and unusable results (affecting 6.0% of all cartridges used for patient testing) were recorded during the study period. Of the 935 errors, 563 (3.6%) were identified as cartridge errors and 372 (2.4%) as unusable results. Across 563 cartridge errors, 156 (27.7%) were associated with error codes related to sample quality or handling issues (e.g. insufficient sample, bubbles in the sample, or over/under filling of the cartridge). Unusable results were observed for 5.7% of all Chem8+ cartridges, 2.1% of all CG4+ cartridges, and 0.6% of all Troponin cartridges. An inverse correlation was found between user experience and error rates. Users who performed <50 tests had a 9.3% error rate in comparison to a 5.6% error rate for users who performed >200 tests during the study period. Testing errors were associated with longer TAT. Cartridge waste (test ordered but not completed) over the two-year period for Chem8+ and Troponin was 15.6% and 11.7% respectively. **Conclusion:** Device errors and incomplete test orders lead to inefficiencies in the POCT process. Although infrequent, device errors can result from multiple root causes, including poor sample quality, inappropriate sample/ device handling and device malfunction, and inversely correlate with user experience. These errors are associated with potentially increased time to definitive diagnosis that impact patient care as well as have economic impact. They lead to higher cost due to demand for additional time, resources and waste of cartridges and consumables. Incomplete test orders also contribute to cartridge waste adding to the economic impact.

B-357

Evaluation of the cobas h232 POCT instrument for NT-proBNP testing

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Background: Nt-proBNP (N-terminal-pro-Brain Natriuretic Peptide) is well-established biomarker for the diagnosis and risk stratification of heart failure (HF). Nowadays, several point of care testing (POCT) are available for testing of cardiac markers and could be used in hôpital settings as well as in ambulatory care. The aim of our study was to evaluate Nt-proBNP testing on the cobas h232® POCT system. **Methods:** The imprecision of the POCT instrument was determined with quality control materials over thirteen consecutive days. Method comparison was performed with our central laboratory Nt-proBNP assay run on cobas 8000® instrument through thirty two serum samples of patients suspected of HF. **Results:** The between-run imprecision coefficients of variation (CV) were 11% and 14% for Nt-proBNP concentrations of 102 and 719 ng/L, respectively. The median concentrations in patients'samples were 798 ng/L (range: 22.3 to 39477) with routine assay and 655 ng/L (60 to 9000) with POCT system. The two Nt-proBNP assays were significantly correlated ($r = 0.97$, $p<0.0001$). The Passing and Bablok regression analysis showed for concentrations between 60 and 6000 ng/L a slope 1.07 (95% CI: 0.87 to 1.21), an intercept of -20.2 (95% CI: -72.2 to 42.9) and no significant deviation from linearity. The Bland and Altman Plots revealed no significant biais and a mean difference of 62.5 ng/L (95% CI: -109 to 234) between the two Nt-proBNP assays. **Conclusions:** Our preliminary data showed that the performances of the cobas h232 Nt-proBNP assay could be compatible with diagnosis of HF as well as monitoring of patients with chronic HF in both hospital and ambulatory care settings

B-358

Impact of audits on point of care test performance in a countywide health system.

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Background: The MetroHealth Health System is a county wide health system with over 50 point of care testing sites. Prior to August 2015, many of these sites had their own CLIA license and lab directors. Pathology provided guidance through two pathology POC staff who performed random checks and helped resolve problems as they occurred. This required each POC site to be responsible for ensuring their own compliance with regulations on a regular basis. There were also no defined ramifications for poor performance. Following an accreditation visit that indicated significant shortcomings, primarily due to the fragmented nature of the POCT program, the POCT program at MetroHealth was unified under the Pathology Department. 27 CLIA certificates were consolidated into four with all CLIAs being held by Pathology personnel. Today all POC testing is performed under 8 CLIA Certificates. The Pathology POC staff was doubled to four which made it possible for them to perform monthly audits. Auditing of these different sites was undertaken to ensure that all regulatory requirements were met and testing was being performed appropriately. **Methods:** : On-site inspections of all sites where POCT testing is done is performed on a monthly basis. The on-site inspection follows the Joint Commission requirements and an audit is generated for each site under the following categories: QC performance and documentation; QC and reagent storage and labeling; Specimen labeling; Adherence to personal protective equipment guidelines; Procedure manual review and availability; Result documentation per protocol; Preventative maintenance performed and documented; Competency testing successfully completed. There is a policy wherein sites that fail any of the categories is notified of the failure; a second consecutive failure or three failures in a five month period in the same category results in a warning notice; and a third consecutive failure or four failures in a six month period can result in loss of testing privileges. **Results:** The number of infarctions identified has decreased from 25 - 30 per month in the latter half of 2015 to about 15 per month. The categories with the most infarctions include QC performance and documentation, QC and reagent storage and labeling, and; Preventative maintenance per-

formed and documented. Most of the infarctions involve documentation issues. **Conclusion:** Auditing POCT sites has enabled detection of problems in real time and enabled resolution of the same in a timely manner. The audits combined with a policy containing defined ramifications for non-compliance has enabled a significant improvement in adherence to regulations and performing tests appropriately which was reflected in the latest accreditation visit (April 2017).

B-359

Evaluation of a Point-of-Care Assay for Fecal Calprotectin

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Background: One of the most useful biomarkers to help diagnose inflammatory bowel disease (IBD) is calprotectin, a primary protein released from neutrophils during an innate immune response. Elevations of fecal calprotectin are suggestive of gastrointestinal inflammation, such as Crohn's disease or ulcerative colitis, and may help rule out idiopathic or mechanical causes for gastrointestinal symptoms. Several clinical assays for fecal calprotectin are available; however, collection and handling of stool samples is unpleasant and patients may be resistant to testing. In response to this problem, several point-of-care (POC) kits have been produced that allow patients to collect, sample, and assay fecal specimens at the time and location of their choosing. While the advantages to the patient are obvious, the performance characteristics of such test kits are not widely reported. Therefore, our objective was to evaluate one POC kit for fecal calprotectin by comparison to 2 immunoassays performed in the clinical laboratory. Methods: 120 residual stool samples were retained from specimens submitted for routine calprotectin testing using the QUANTA Lite™ ELISA (Inova Diagnostics). Specimens were stored frozen (-20C). Calprotectin was measured using the POC QuantOn Cal kit (Immundiagnostik, Bensheim, Germany) according to package instructions. This kit utilizes a lateral flow immunochromatographic cartridge with a proprietary smart-phone app to read and quantify color change. To provide an additional comparison, calprotectin was also measured using the IDK Calprotectin ELISA (Immundiagnostik). In addition to method comparisons, precision was assessed for each assay across the measuring range. Package inserts for each assay stated normal calprotectin concentrations as <50 mcg/g. Cutoffs for positive inflammation were: Inova, >120 mcg/g; IDK POC, >100 mcg/g; IDK ELISA, >200 mcg/g. In each assay, the range between normal and positive is considered indeterminate. Results: Overall qualitative concordance between the POC device and the Inova assay performed in our laboratory was 49% (Kappa = 0.27) when using normal, indeterminate, and positive categories. Using only the positive cutoff for each assay, concordance increased to 70% (Kappa = 0.41). Among results within respective analytical measuring ranges, Passing-Bablok regression was: POC = 3.24*Inova + 1.84; r = 0.274. Concordance between the POC and IDK Calprotectin assays (produced by the same company), was 61% (Kappa = 0.39) using all three ranges, and 75% (Kappa = 0.50) using only the abnormal cutoff. Passing-Bablok regression was: POC = 1.76*IDK ELISA + 1.70; r = 0.362. Precision for the POC device was calculated at 37% (64 mcg/g, n=20), 41% (166 mcg/g, n=18), and 23% (588 mcg/g, n=20). In contrast, inter-assay CVs for the IDK ELISA were 16% (32 mcg/g), 12% (75 mcg/g), and 11% (464 mcg/g). For the QUANTA Lite ELISA, inter-assay CVs were 7.6% (14.2 mcg/g), 6.0% (88.5 mcg/g), and 7.4% (525.9 mcg/g). Conclusions: Calprotectin measurements collected by the POC device tested in this study showed only moderate qualitative and quantitative concordance with 2 laboratory immunoassays. Discrepancies between results are largely in the borderline range and may partially be attributed to the imprecision of the POC device.

B-360

Performance Evaluation of the Atellica UAS 800 urine particle Assay

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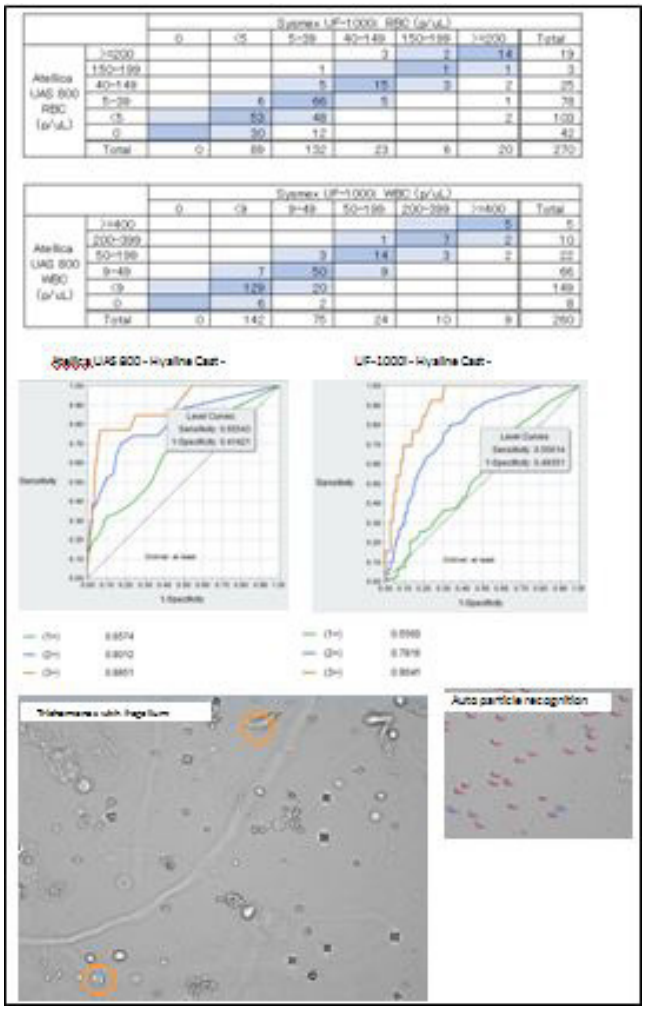
Background: Urine Microscopy testing is commonly performed in clinical laboratories to help identify kidney and urinary tract diseases. However, manual microscopy testing is known to be time-consuming, labour intensive and most often, subjective. In this study, we evaluated the performance of the Atellica UAS 800 (UAS 800) urine particle analyzer (by Siemens). **Method:** UAS 800 is an automated urine particle analyzer powered by high-resolution digital imaging designed to minimize the need for manual microscopy testing. It recognizes, counts and classifies particles into 11 major categories as Bacteria, Crystals, Hyaline Cast, Mucus, Non-squamous EC, Pathological Cast, RBC, Squamous EC, WBC, Sperm and WBC clumps using a reference library built based on over 110,000 particles and a dual-focusing mechanism to produce clear images. 270 freshly collected urine samples submitted to our hospital laboratory were analyzed with UAS 800 and UF-1000i (by Sysmex) . **Results:** For RBC, the concordance rate between UAS 800 and UF-1000i are 55.2%(Exact Agreement), and 92.2%(±1 Block Agreement). For WBC, the concordance rate between UAS 800 and UF1000i are 78.8% (Exact Agreement), 98.5%(±1 block agreement). RBC and WBC cell recognition by both, UAS 800 and UF-1000i is considered to be equivalent. Hyaline Cast data shows a higher sensitivity on UAS 800 than UF-1000i. A well-received advantage for using digital technology, such as UAS 800, is the availability of clear full-field view of images which helps to reduce Manual Microscopy and to timely identify samples that may require further testing. It was the first experience to confirm flagellum of Trichomonas using an automated urine analyser. **Conclusions:** As a screening method, UAS 800 with its clear full-field view of images is expected to reduce the manual microscopy rate.



B-361

Electrochemical detection of Parathyroid hormone as a point-of-care testing device towards clinical applications

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Background: Every year, about 100,000 people develop primary hyperthyroidism in the United States, making it one of the most common endocrine disorders. Enlargement of one or more of the parathyroid gland is seen in 70% of all patients due to overactivity of the affected gland. Measuring parathyroid hormone (PTH) levels helps in the investigation and management of patients with parathyroid disorders. Reliable

treatment includes surgical excision of the hyperfunctioning gland, where successful resection is confirmed by measuring PTH levels. Current lab-based methodologies are time-consuming, require larger sample volume and dedicated laboratory facilities. To overcome current drawbacks, it is advantageous to develop a point-of-care device that reports PTH concentration in real time, requires small sample volume, and minimal training. The objective of this study was to develop a user-friendly biosensor for highly sensitive and rapid detection of PTH using ultra-low sample volume of human serum and whole blood. **Methods:** We have developed an affinity-based electrochemical biosensor that contains nanostructures selectively grown on the sensor electrode surface. This helps in a size based matching environment for the biomolecules and enhances sensitivity. The immunosensor consists of anti-PTH antibody directed towards 1-84 intact molecule, as a capture probe bound to the electrode surface using dithiobis succinimidyl propionate which is a thiol-terminated crosslinker. The sensor response for the targeted PTH analyte in the range from 1 pg/mL to 1 ng/mL was evaluated using an electrochemical technique where binding of PTH on the sensor results in frequency dependent impedance change. Cross-reactivity of the sensor-antibody was tested against adrenocorticotropic hormone (ACTH), parathyroid hormone-related protein (PTHrp) and cortisol to measure the selectivity of the sensor to PTH molecule. Sensor performance was evaluated across 8 replicates in both human serum and whole blood. **Results:** Biosensor surface characterization was performed using scanning electron microscopy to confirm uniform deposition of nanostructures for obtaining maximum sensor response. Quantification of PTH concentration (1 pg/mL-1ng/mL) in human serum and whole blood were assessed using change in impedance. Noise threshold for the biosensor was calculated as three times the standard deviation of blank over baseline impedance value. The biosensor demonstrated 10 pg/mL as the limit of detection with a dynamic range from 10 pg/mL to 1 ng/mL in the clinically relevant measurement range. Cross-reactivity of ACTH, PTHrp and cortisol were within the calculated noise threshold of the sensor, thereby indicating the specificity of the electrochemical biosensor. Furthermore, the response time of the sensor was less than 15 minutes. **Conclusion:** We developed an affinity-based electrochemical biosensor by leveraging semiconducting nanoscale properties for detection of PTH molecule. Using electrochemical based impedance response, we have quantified PTH concentrations (10 pg/mL- 1 ng/mL) in human serum and whole blood. We have also demonstrated a fast response time for detection of PTH using 40 µL sample volume. The sensor showed a high degree of specificity and sensitivity to PTH molecule. These results from the preliminary data in both human serum and whole blood samples show a proof-of-translatability as an ideal point-of-care diagnostic device for PTH detection, real-time in a clinical setting.

B-362

The Thromboelastography G Parameter (TEG-G) For Predicting Onset of Acute Coronary Syndrome (ACS)

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Background: The most prominent event that defines an ACS is the formation of an intra-arterial thrombus, usually resulting from activation of platelet and fibrinogen in coronary arteries at the site of a ruptured plaque with physical lumen occlusion by thrombosis. The aim of this study was to investigate whether TEG (a POCT method) parameters could be surrogate markers of thrombus formation process and diagnosis of ACS. **Methods:** A total of 168 patients with ACS, 58 patients with stable angina pectoris (SAP) as control were enrolled. Baseline characteristics were recorded. Routine blood test, cardiac markers, routine coagulation tests and TEG were determined. Receiver operating characteristic (ROC) curve was used to evaluate the diagnosis performance of each index. Logistic regression analysis was used to define the independent risk factors of ACS. **Results:** Patients with ACS exhibited greater prevalence of hypertension than patients with SAP (p<0.01). cTNI and NT-proBNP levels in SAP measured were significantly lower than ACS patients (p<0.01). PT was significantly different in two groups, while FDP was significantly increased in ACS patients (p<0.05). FIB, D-dimer and PLT were greater elevated in ACS patients than SAP patients (p<0.01). Most parameters (K, Angel, MA, CI and G) of TEG have significant difference between two groups(p<0.01), except R value. Logistic regression analysis showed that TEG-G was an independent risk factor and auxiliary diagnostic indicator for ACS (odds ratio [OR], 2.760; 95% confidence interval [CI], 1.939-3.928). The area under ROC curve of TEG-G was 0.899. The optimal cut-off value for the diagnosis of ACS was 9.95 dyne/cm^2, while the sensitivity was 80% and the specificity was 87.9%. **Conclusion:** TEG-G could be used as a better predictor of activation of platelet and fibrinogen in force unit than MA, which is eligible to be a new biomarker for early diagnosis of ACS and could provide baseline information for anti-platelet therapy.

Table 1. Receiver operation characteristics (ROC) curve parameters

	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)
cTNI (ng/ml)	0.743 (0.676-0.810)	0.04	57.0	82.8
NT-proBNP (pg/ml)	0.739 (0.667-0.811)	150.35	72.1	69.0
D-dimer (mg/L)	0.656 (0.575-0.737)	0.36	53.9	74.1
FIB (g/L)	0.783 (0.717-0.850)	2.85	77.0	70.7
PLT (×10^9/L)	0.687 (0.614-0.760)	202.00	45.0	88.5
ANGLE (degree)	0.823 (0.764-0.882)	69.40	66.1	89.7
MA (mm)	0.899 (0.855-0.943)	66.6	80.0	87.9
CI	0.814 (0.754-0.875)	1.55	68.5	82.8
G (dyne/cm^2)	0.899 (0.855-0.943)	9.95	80.0	87.9

B-363

Preventing Blood Loss and Iatrogenic Anemia from Diagnostic Testing; A Laboratory Medicine Best Practices Systematic Review

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Objective: The CDC's Laboratory Medicine Best Practices initiative (LMBP™) conducts systematic reviews to assess the effectiveness of quality improvement practices. With a panel of experts from relevant laboratory and healthcare disciplines, and scientists from RTI, we reviewed practices for preventing blood loss and reducing the occurrence of iatrogenic anemia from diagnostic testing, especially in critical care patients. As many as 90% of patients develop anemia by their third day in an intensive care unit (ICU). Practices to reduce blood loss are important to patients' health and survival. **Methods**. Employing the A-6 methodology[1], developed by the Centers for Disease Control and Prevention's LMBP™, searches of PubMed, Embase, Cochrane, Web of Science, PsychINFO, and CINAHL retrieved 2,564 abstracts. Twenty-one studies were accepted for full text review based on A-6 criteria. Five interventions were reviewed: (1) small volume tubes, (2) closed blood sampling devices, (3) point of care testing, (4) educational interventions, and (5) bundled interventions that variously combined two or more interventions. The overall strength of the body of evidence was rated with respect to supporting recommendations for specific practices (or not) and categorized as High, Moderate, Suggestive, or Insufficient as defined by the A6 methodology.[1] **Results**. We found moderate strength, consistent evidence that blood conservation devices returning blood from venous and arterial lines to the patient reduced blood loss by approximately 25% in both neonatal ICU (NICU) and adult ICU patients. The effect estimate (mean difference) by meta- analysis was 24.7 (95% CI = 12.1 - 37.3). The evidence was suggestive that bundled interventions that included such blood conservation devices also reduced blood loss by at least 25%. However, the evidence was insufficient to conclude that these devices reduced hemoglobin decline or anemia risk. There was suggestive evidence that use of small volume phlebotomy tubes may reduce blood loss, but insufficient evidence to evaluate the impact on hemoglobin levels or transfusion rates. (Suggestive evidence is not sufficient for LMBP™ to make recommendations.) **Conclusion**. Closed blood conservation systems were effective in reducing blood loss in ICU and NICU patients. The evidence is moderate strength that such devices reduce blood loss by about 25% compared to patients with conventional arterial pressure monitoring systems. Thus, the LMBP™ recommends the use of blood conservation systems with arterial or venous catheters to eliminate blood waste when drawing blood for testing. Additional high-quality studies and evidence are needed to adequately assess several other commonly proposed interventions to reduce blood loss from diagnostic testing among critically ill patients.

1. Christenson RH, Snyder SR, Shaw CS, et al. Laboratory medicine best practices: systematic evidence review and evaluation methods for quality improvement. Clin Chem. Jun 2011; 57(6):816-825.

B-364

"Just-in-Time" Implementation of Molecular Point-of-Care Testing for the 2017-18 Influenza Epidemic

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Background: The ability to timely implement next generation testing to aid in diagnosis and treatment during current health crises is critical for any institution. "Just-in-time" planning optimizes resource allocation while ensuring maximum benefit. The 2017-18 influenza season has proven challenging for health care providers given the unanticipatedly high prevalence of the H3N2 viral strain. According to the Center for Disease Control, between October 1, 2017 and January 27, 2018 approximately 15,000 laboratory confirmed influenza-associated hospitalizations have occurred. Many hospitals rely on antigen-based rapid influenza detection tests (RIDT) for screening of influenza infection. While RIDTs provide quick results (<15 minutes), their poor sensitivity has resulted in the United States Food and Drug Administration reclassifying them as Class II devices effective January 2018—forcing many institutions to find alternative methods. To meet this need, our goal was to conduct "just-in-time" implementation of a point-of-care (POC) molecular assay for viral detection during the 2017-2018 influenza season. **Methods:** Thirty POC molecular analyzers (cobas Liat, Roche Diagnostics, Indianapolis, IN) analyzers were obtained for 16 of our health system clinics, the emergency department (ED), and the clinical laboratory. Precision was assessed on all 30 instruments for the influenza A/B assay by analysis of two-level within day (n=5) testing and two-level daily testing for ten days. The same precision testing scheme was performed on a subset of instruments (n=4) to validate the combined influenza A/B and Respiratory Syncytial Virus (RSV) assay for use in the ED and clinical laboratory. In addition, de-identified positive and negative patient universal transport media samples for influenza A/B, and RSV were tested on the molecular POC analyzer and compared results to a predicate analyzer (GenMark Diagnostics, Carlsbad, CA). An educational program was implemented to train 562 device users and provide proper test utilization for molecular testing. **Results:** The POC molecular influenza A/B assay demonstrated sensitivities of 100% [Flu A (6/6); Flu B (4/4)] and a specificity of 100% [Flu A (15/15); Flu B (17/17)] for each target when testing remnant patient samples for method comparison. Likewise, the influenza A/B and RSV samples showed 100% positive (7/7) and negative (13/13) agreement for each target across four instruments. Of the 1,020 two-level controls (positive and negative) tested across the 30 instruments during precision analysis, all tests yielding a result corresponded correctly. We determined inadequate mixing as a common source of pre-analytical error and incorporated these findings into the waived user training scheme. Operator education began in parallel using a "train the trainer" model coupled to laboratory test best practice notifications for physicians. Total time from commencement of performance verification to clinical implementation was 64 days. Post-implementation results show no statistically significant changes in ordering practices and only two reported cases of pre-analytical error requiring repeat testing. **Conclusion:** The Roche cobas Liat has high precision and clinical sensitivity specificity for the Influenza A/B, and combined Influenza A/B and RSV assays. Implementation and distribution of molecular POC testing to clinics and emergency departments can be completed using a "just-in-time" model to respond to current public health crises while optimizing resource utilization.

B-365

The importance of health economics modeling in assessing costs of point-of-care HbA1c testing of patients with diabetes mellitus type II in the United States

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OBJECTIVES: To show the importance of health economics modeling when assessing costs of HbA1c testing and to calculate the financial impact of the point-of-care methodology in the United States. **METHODS:** We developed a budget-impact model (BIM) that compared strategies of using point-of-care (POC) versus conventional laboratory-diagnostics (LD) HbA1c testing in patients suffering from diabetes mellitus (DM) type II. In BIM, we followed a cohort of 2,900,000 patients diagnosed with DM type II in the United States for the period of 15 years and estimated the costs of complications (amputation, cataract extraction, kidney failure, heart failure, stroke, and microvascular disease) using the local data. To assess the validity of the assumptions and robustness of the model, a thorough sensitivity analysis was undertaken. **RESULTS:** In patients with DM type II, POC HbA1c testing resulted in the saving of $2.824 billion (on average, $974 per patient in the cohort) when compared to conventional LD testing. The sensitivity analysis showed robustness of our findings. **CONCLUSIONS:** Our health economics analysis predicts that the POC HbA1c testing in patients suffering from DM type II may reduce overall heath care costs in the United States. This finding has important potential implications for management of the diabetic population and reimbursement of HbA1c testing methodologies.

Wednesday, August 1, 2018

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

Proteins/Enzymes

B-366

Diagnostic Performance of Enhanced Liver Fibrosis (ELF) test in Predicting Liver Stiffness

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Background: Fatty liver disease is not uncommon in general population. It represents a wide spectrum of pathologic findings, from simple steatosis to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. The diagnostic assessment of liver fibrosis is an important step not only in the management of patients with chronic liver diseases but also in the assessment of the true burden of liver disease in the general population. Liver biopsy is considered the gold standard for the assessment and quantification of liver fibrosis. But noninvasive techniques including serum biomarkers have been developed to circumvent the need for liver biopsy. Therefore, the aim of our study is to assess the diagnostic performance of enhanced liver fibrosis (ELF) test in predicting liver stiffness by using magnetic resonance elastography (MRE) as a reference standard in health checkups. **Methods:** This study included 89 health examinees who underwent MRE and ELF test at health promotion center in Korea, between July 2016 and December 2016. ELF score was compared with MRE results. Receiver operating characteristic (ROC) curve analysis was performed for ELF test as a predicting test for liver stiffness. **Results:** Area under ROC (AUROC) to predict mild liver stiffness, and moderate-to-severe liver stiffness were 0.613, and 0.891 for ELF test. Optimized cutoffs of ELF to maximize sum of sensitivity and specificity were 8.98, and 10.3 for mild stiffness, and moderate-to-severe stiffness, respectively. **Conclusions:** ELF test demonstrated considerable diagnostic value in predicting liver stiffness in health checkups.

B-367

Targeting TMAO Biosynthesis. Discovery of New Novel TMA Lyase Inhibitors to Protect Atherosclerosis lesion, MI and Stroke

A. S. Duzan. *Cleveland state university, Westlake, OH*

Background: Recent clinical research evidence has marked TMAO as a biomarker molecule associated with several homeostasis disruptions, such as myocardial infarction, atherosclerosis, secondary hypertension, irritable bowel syndrome, chronic kidney disease, strokes, and heart failure. The priority is to stop the biosynthesis pathway of TMAO through inhibition gut microbial TMA lysate CutC/D. Trimethyl amine (TMA) formed in the gut from choline after breaking by anaerobic gut bacteria CutC/D lyase. TMA affects the microbiota environment and human health. TMA is transported to the liver and then is metabolized by flavin monooxygenase 3 (FMO3) to trimethylamine-N-oxide (TMAO). TMAO works as an independent atherogenic factor via increasing macrophages hyperactivity and lowering high density lipoprotein (HDL) level that leads to myocardial infarction, stroke and death. In fact, structure activity relationships (QSAR) provide the ability to predict potent inhibitors. **Methods**: Our analogue synthesis was designed by reacting different electrophilic groups to simple analogous. The synthesis was conducted through a sequence of reactions by nucleophilic substitution reactions, and sometimes de-esterification, amination, alkylation, and sulfonation. After completion the synthesis, characterization and purification process using HRMS, NMR (^{13}C,^{1}H,^{15}N) and FTIR. The biological specific activity measurements of CutC/D enzyme have done for each compound in vitro and in vivo screens using different gut bacterial species and female mice, and quantified it by using AB Sciex QTRAP 5500 LCMS-MS. The leading and the potent inhibitor will be used further for pharmacokinatic measurements. The mechanism of inhibition of the leading compounding was confirmed by time dependent inhibition assay, irreversibility assay, and the measured change in Km & Vmax over using different concentrations. **Results**: We describe the design, synthesis, chemical characterization, and the biological activity of inhibitors derived CutC inhibitor give an IC_{50} in Nano molar range (3 orders of magnitude lower than 3,3-dimethyl-1-butanol, "1stgeneration") (Wang.Z. Cell.2013) in vitro screen, and it shows a high potency in vivo screen. Moreover, our data shows potential results; (1). Synthesis of potent inhibitors that has highly efficacy in vitro and in vivo study; (2). Non-lethal effect on gut microbial community; (3). The leading inhibitor works as irreversible non-competitive inhibitor to get high potency and fewer side effects. (4) Our inhibitor possesses physic-chemical pharmaceutical properties as necessary for drug effect; (5). The leading compounds do show acceptable pharmacokinetic/ pharmacodynamics properties. **Conclusion**: This study shows that inhibitor like-substrate analogous gives a single digit Nano molar inhibition concentration in vitro screen and works irreversible noncompetitive inhibition of TMA lysate CutC/D without any cytotoxicity on gut microbiota or on mice. Docking helps to design and synthesis universal inhibitor that can stop cleaving choline from other species of gut microbiota. Complementary docking studies of this inhibitor to the crystal structure of the CutC enzyme point to specific inhibitory effects of this type of compound and pave the way for further optimization of the chemical structure to increase further the inhibitory potency of this class of compounds.

B-368

Interference of daratumumab in the measurement of the monoclonal peak in patients with multiple myeloma

F. Barrero Alor, Á. Gragera-Martínez, A. León-Justel, I. Vázquez Rico. *Juan Ramon Jimenez Hospital, Huelva, Spain*

Background:
New therapies with monoclonal antibodies are being used more and more in the face of different pathologies. In the case of multiple myeloma, the use of Daratumumab constitutes a therapeutic advance and it is expected that in the future more and more drugs based on monoclonal antibodies will be commercialized. Daratumumab is a human monoclonal antibody IgG1k against the antigen CD38 produced in a Chinese hamster cell line that is being used in the treatment of multiple myeloma. We have a first patient in treatment with this drug in which we consider how to follow up during the treatment. The quantification of the monoclonal peak by capillary electrophoresis is useful for the clinician to see the tendency of the patient's response to treatment, increase and decrease. In IgG myeloma we can not know if the monoclonal peak we are seeing is due to myeloma or daratumumab. In addition, when quantifying the monoclonal component we can not know how much of that peak is due to the monoclonal component and how much to the IgG of daratumumab, making this measurement difficult.
Methods:
We use capillary electrophoresis (Capilarys®, Sebia) for the detection and quantification of the monoclonal component. The identification of the monoclonal component is done by immunofixation (Hydrasys®, Sebia). This is a patient in which there is no monoclonal peak in the graph of capillary electrophoresis and therefore an immunofixation is carried out. In the immunofixation a monoclonal band is observed, we can not differentiate if it is a band due to the drug or because the patient continues to have a monoclonal band due to multiple myeloma. The use of immunocomplex daratumumab / anti-daratumumab (Hydrashift®, Sebia) is proposed to perform immunofixation allowing the differentiation of the monoclonal component and the band due to the drug.
Results:
Once immunofixation was performed using the daratumumab / anti-daratumumab immunocomplex, the disappearance of the monoclonal band was observed, due to the drug and not to the progression of the disease. Therefore, the patient remained in complete remission, did not present a monoclonal component and serum free light chains were normal.
Conclusion:
The use of Hydrashift® (Sebia), although expensive, may be a solution at this time. We consider what will happen when other drugs based on monoclonal antibodies come out. It would be very expensive to have kits for each drug. At the moment we only have one patient in treatment with daratumumab. The option would be to send the sample to a centralized external laboratory that is in charge of immunofixing when these types of drugs are used. This is still necessary because, according to clinical guidelines, immunofixation is a necessary test for the assessment of response to treatment. On the other hand, the use of free light chains could be a more versatile alternative in the case of patients with multiple myeloma treated with monoclonal antibodies.

B-369

Comparisons of Serum Albumin Measurement by Chemistry Analyzer and Electrophoresis

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Background: Serum protein electrophoresis (SPEP) is a widely available and inexpensive laboratory test that examines specific serum proteins based on their physical properties. Albumin and five major globulin fractions are identified. In clinical practice, SPEP is indicated when multiple myeloma, macroglobulinemia, amyloidosis, or other protein disorders are suspected. After the SPEP is completed, a densitometric scan of the electrophoresis pattern is performed. Numeric values for each fraction can be obtained based on the percentage of serum total protein, including albumin. Some patients may have an albumin that resulted from the chemistry analyzer (bromcresol green dye) as well. This study is to compare the albumin results from two different methodologies. The findings may prevent duplicate orders and promote cost saving. **Methods:** A total of 214 serum samples were measured for albumin by the bromcresol green dye (Abbott Architect) and amidoblack dye (Sebia Hydrasys 2), ranging from 1.25 to 5.33 g/dL. Comparison analysis was perform in Microsoft Excel. **Results:** Comparison of results from both methods showed high correlation (R^2 = 0.923) with electrophoresis results 20% higher than the chemistry analyzer results. The mean of the chemistry analyzer results was 3.66 g/dL (n = 214), compared to the mean of the electrophoresis results at 3.69 g/dL, demonstrating no clinically significant difference. **Conclusion:** In summary, the albumin results from SPEP, although an estimation and analyzed in an indirect way, are comparable clinically to those from a standard chemistry analyzer. Clinicians should be informed with this finding and be mindful about ordering lab tests. Preventing duplicate albumin orders could save the cost in a long run.

B-370

Evaluation of the reference methods of alkaline phosphatase

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Background: Alkaline phosphatase (ALP or AKP) is widely distributed in human liver, bone, intestine, kidney and placenta and other tissues, and it's of great significance in the clinical diagnosis.And ALP is the very important routine test in serum enzymes determination. Diagnosis,treatment and prognosis of diseases are subject to the accuracy of the test. In consideration of it,this research bases on the reference method recommended by IFCC,to evaluate the performance of the reference methods of alkaline phosphatase (ALP). **Methods:** The reference method was established according to the primary reference procedure for the measurement of catalytic activity concentration of ALP(37°C),which had been published by IFCC. Furthermore, the EP5-A2 and EP6-A protocols were used for evaluation of the precision and linearity range of the methods. Because there is no ALP reference materials in current JCTLM(Joint Committee for Traceability in Laboratory Medicine) list, the samples of RELA[International Federation of Clinical Chemistry and Laboratory Medicine(IFCC) external quality assessment scheme for reference laboratories in laboratory medicine] were used for verification of the equivalence. **Results:** Ultraviolet spectrophotometer, analytic balance, pH meter, electronic thermometer,pipettes and volumetirc flask have been verified and its uncertainty fit to method's requirements.When the temperature controller showed 37.6°C,the temperature in cuvette was equal to 37.0°C. The temperature in cuvette reaches the set temperature after 180 seconds.The CV_r%(The imprecision within run) and CV_T %(Total imprecision) were less than 1%. The results of the RELA within the limits of equivalence provided by IFCC. They can verified the precision and trueness of the reference method. The upper limits of the measurement ranges of ALP were 610U/L. **Conclusion:** The reference method has been established, which can be used widely for reference measurement service and correlative standardization research.

B-371

Transference of CSF Total Protein Reference Intervals from the Siemens Vista to the Ortho VITROS

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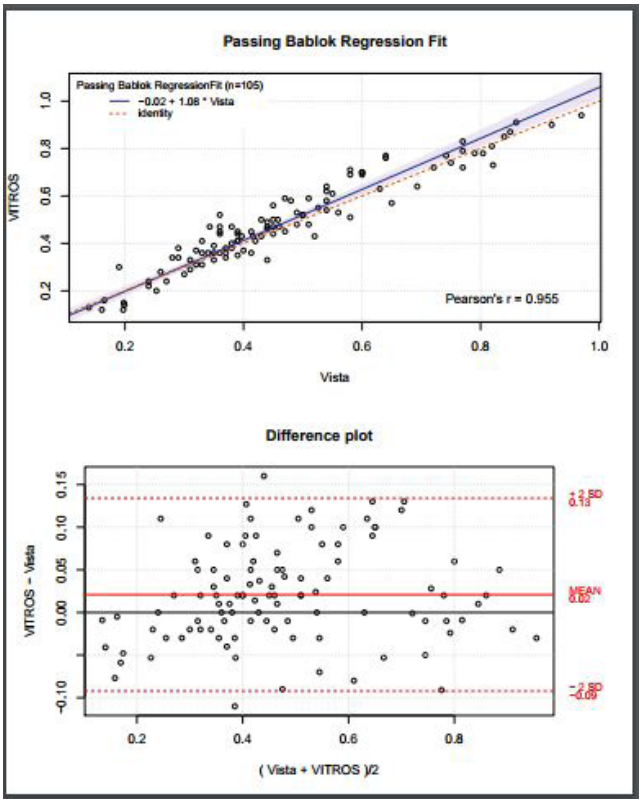
Background: Reference intervals are vital for interpretation of laboratory results. Many existing reference intervals for CSF total protein (CSF-TP) are derived from old literature because of the invasive nature of sampling. The objective of this study was to transfer reference intervals for CSF-TP from a previously validated instrument (Siemens Vista) to the Ortho Clinical VITROS. **Methods**: One hundred and thirty-three CSF samples ordered for CSF-TP testing were compared between the Siemens Vista 1500 and the Ortho Clinical VITROS 5,1; the Vista method uses pyrogallol red and sodium molybdate measured at 600 nm whereas the VITROS method uses a copper-azo dye complex measured at 670 nm. Samples were remaining waste from collection for clinical use and included 100 adults and 33 children aged 0-18 years of age. Values that were below the measuring limit of either method were excluded (n=3, <0.05 g/L Vista; <0.1 g/L VITROS). CSF-TP values >5 g/L were also excluded as they would all be 5x higher than the highest reference value (n=22). Analysis was performed with (n=115) and without (n=105) outliers defined as values >1.5 the interquartile range. Passing-Bablock regression and difference plots were used to compare methods. Bootstrap resampling was used to calculate confidence intervals for the slope and intercept estimates. **Results**: CSF-TP results were similar between instruments, with a slope of 1.09 (1.00-1.19) and y-intercept of -0.021 (-0.063-0.020; Pearson's R=0.85) for all samples; after removal of outliers, the slope was 1.08 (1.00-1.17) and y-intercept was 0.017 (-0.056-0.020) with the same intercept after removal of outliers (Pearson's R=0.96). There was mean bias of 0.02 g/L (-0.19-0.23) for the VITROS with and without outliers. **Conclusions**: The VITROS CSF-TP method agrees well with the Vista method. This indicates that the recently determined age and sex partitioned interval is application to the VITROS method.



B-372

Development of an Assay for Measurement of Transferrin (TRSF) in Urine Samples on Roche Clinical Chemistry Analyzers

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Background:
Transferrin is a glycoprotein with a molecular weight of 79570 daltons. It consists of a polypeptide strand with two N-glycosidically linked oligosaccharide chains and exists in numerous isoforms.1,2 The rate of synthesis in the liver can be altered in accordance with the body's iron requirements and iron reserves.Urinary transferrin is considered as a biomarker of glomerular nephropathy with high association to albuminuria. Quantitation of urinary transferrin, when used in conjunction with albumin results, permits an estimation of the charge selectivity of glomerular defects as both proteins are of similar size but different charge. In diabetic patients, urinary transferrin is discussed as an earlier marker of glomerular damage than urinary albumin and increased urinary transferrin excretion might precede the development of microalbuminuria and more advanced tubulointerstitial lesions.
Methods:
Immunoturbidimetric assay.Human transferrin forms a precipitate with a specific antiserum which is determined turbidimetrically.
Development Goals:
-Development of an application for analysis of urine samples based on the existing Tina-quant® Transferrin Ver.2 assay
-Measuring range: ≥ 2.2 - 35.0 mg/L
-In use time Transferrin reagent: 8 weeks (cobas c pack), 4 weeks (cobas c pack large)
-Low sensitivity to drug interference.
Results:
The linear assay range of the TRSFU assay is 2.2 - 35.0 mg/L. Extended measuring range: 35.0 - 105 mg/L.
Limit of Blank: 1.0 mg/L, Limit of Detection: 1.5 mg/L, Limit of Quantitation: 2.2 mg/L with 20 % total error.
TRSFU assay has been standardized against the ERMDA470k/IFCC standard. Precision - CLSI EP5 - 21 days (Repeatability): SD ranging from 0.09 mg/L to 0.25 mg/L, CV ranging from 0.7% to 2.3%, sample concentration range: 4.4 mg/L to 33 mg/L. Method comparison study: Human urine samples obtained on a Roche/Hitachi cobas c 311 analyzer (y) were compared with those determined using the corresponding reagent on a Roche cobas c 501 analyzer (x) (n = 80). Passing/Bablok regression: y = 1.044x - 0.0461 mg/L. The sample concentrations were between 2.46 mg/L and 32.8 mg/L.
Conclusion:
All of the development goals for the urine application of Roche Tina-quant® Transferrin assay were met. The introduction of TRSF urine assay will complete the assay portfolio for screening and monitoring patients with kidney disease.

B-373

Validation of interleukin-5 (IL-5) and interleukin-9 (IL-9) multiplex electrochemiluminescence immunoassay

W. Obeid, D. G. Moledina, J. M. El-Khoury, C. R. Parikh. *Yale School of Medicine, New Haven, CT*

Background: Interleukin-5 (IL-5) and interleukin-9 (IL-9) are produced by type 2 immune responses, and regulate eosinophil migration into tissues, amplify allergic immune responses. IL-5 and IL-9 are being investigated as biomarkers of drug allergies affecting the kidneys. However, data on validation of these assays in the urine is currently not available. Our objective was to develop and validate a multiplex immunoassay panel for measuring IL-5 and IL-9 in urine
Methods: We developed an electrochemiluminescent (ECL) multiplexed assay to measure IL-5 and IL-9 in urine on the Meso Scale Discovery (MSD) U-Plex platform, which allows for flexible multiplexing of immunoassays while requiring a small volume (50uL) of sample. Briefly, the biotin congregated IL-5 and IL-9 capture antibodies were coupled to two different unique linkers. The linker coupled antibodies then assemble themselves on different spots in the U-Plex plate in each sample well. After analytes in the sample bind to their respective antibodies, detection antibodies coupled to SULFO-TAG ECL labels are added to complete the sandwich immunoassay. Once the binding is complete, the concentrations are measured by MESO QUICKPLEX SQ 120 instrument. To validate IL-5 and IL-9 assays in MSD, we performed dilution linearity and spike-recovery experiments. We investigated dilution linearity by performing serial dilutions at three levels (Neat, 1:2, 1:4) assayed in triplicate. We defined acceptance when %CV was less than 5% and recovery was between 80% - 120%. We performed spike and recovery experiment at three different concentration levels (High, Low, and assay diluent) by adding 5% spike volume of mixed IL-5, IL-9 calibrator or assay diluent to the samples. We defined acceptance when recovery was between 80% - 120%. We determined precision using two levels of QC over three days.
Results: IL-5 had a recovery of 83 - 94 %, intra-assay precision of 5.8 %, and a dynamic range of 0.10 to 2080 pg/mL. IL-9 had a recovery of 90 - 97 %, intra-assay CV of 4.1% and a dynamic range of 0.027 - 580 pg/mL. The samples did not require dilution to achieve acceptable recoveries.
Conclusion: We demonstrate that IL-5 and IL-9 can be routinely measured in urine samples using this duplex assay without the need for dilution and with minimal sample preparation.

B-374

Performance evaluation of the general chemistry panel on the Alinity c system

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Background: Abbott General Chemistry solutions offer a broad menu of optimized Six Sigma quality chemistry assays that deliver consistent, comparable results across harmonized systems allowing evaluation of over one hundred analytes. The Alinity ci system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 1350 tests per hour. The Alinity ci system has an increased reagent load capacity, holding up to 70 Clinical Chemistry reagents, onboard QC and calibrators, clot and bubble detection ability, and smartwash technology to provide consistent and reliable results.
Objective: To demonstrate the analytical performance of representative assays from the General Chemistry Panel of the Alinity c system, which consists of assays that utilize photometric technology for the quantitative determination of analytes in human serum, plasma, or urine.
Methods: Key performance testing including precision, limit of quantitation (LoQ), linearity, and method comparison were assessed per Clinical and Laboratory Standards Institute (CLSI) protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met.
Results: The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the General Chemistry Panel are shown in the table below.

Assay	Total %CV	LoQ	Method Comparison to ARCHITECT (Slope/r)	Measuring Interval
Pancreatic Amylase	≤ 3.6	2 U/L	0.99/1.00	2 to 2200 U/L
Total Bile Acids	≤ 1.2	0.2 µmol/L	1.01/1.00	1.0 to 180.0 µmol/L
Cholinesterase	≤ 0.9	115 U/L	0.99/1.00	164 to 25,000 U/L
Dibucaine	≤ 2.3	83 U/L	1.00/1.00	83 to 4,000 U/L
UIBC (Unsaturated Iron Binding Capacity)	≤ 3.3	25 µg/dL	1.00/1.00	25 to 500 µg/dL

Conclusion: Representative clinical chemistry assays utilizing photometric technology on the Alinity c system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT clinical chemistry assays.

B-375

Development of a calibration verification Kit for Analytes in a Synthetic Body Fluid Matrix.

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Introduction: Body fluids are non-vascular liquids normally produced by the human body. Examples include cerebrospinal, peritoneal ascites, pleural and pericardial fluid. Accumulation of these fluids at abnormal levels can be an indication of serious pathological problems. Typically, samples of these body fluids are collected for analysis with methods that are validated for serum, plasma or urine matrices. In 2009-2010, validation of "alternate specimens," which includes body fluids, was implemented in

the CAP checklist. It is required to perform calibration verification for these analytes in a body fluid matrix or its equivalent. Our objective was to develop a liquid-stable, multi-constituent calibration verification and linearity test kit in a synthetic body fluid matrix that represented each individual assay's performance in native fluid in order to meet the clinical laboratory's need for method validation of assays used to test and report results for body fluid specimens. **Methods:** VALIDATE® Body Fluids was prepared with a synthetic body fluid matrix. Each of twelve analytes, albumin, amylase, CA19-9, CEA, cholesterol, creatinine jaffe, glucose, lactate, lactate dehydrogenase, total protein, triglycerides and urea nitrogen, were added to the matrix at individual, predefined concentrations. Recovery targets were optimized to align with the analytical measuring ranges (AMR) for the Roche cobas® 6000. Five levels, with equal-delta concentrations between consecutive levels, were formulated according to CLSI EP06-A. For each level, samples were tested in triplicate. Reported recoveries were evaluated for mean, SD and linearity using MSDRx® (LGC Maine Standards' proprietary linearity software). Limits were applied as 50% of the total allowable error (TAE) for the analyte. To establish product stability claims, samples were subjected to one freeze-thaw cycle, followed by a 72 hour, 30ºC stress condition and four subsequent open-vial events. Real time and stress stability testing is on-going. **Results:** All analytes were determined to be linear and within applied TAE limits through the validated range reported for the Roche cobas® 6000. Linear regression analysis of theoretical vs recovered, for example, glucose was $Y = 0.991\ X + 2.043$, $R^2 = 0.9997$. Other analytes demonstrate similar results. A comparison against the reportable range of the Roche cobas® 6000 demonstrates full coverage for each analyte in the body fluid matrix. All analytes were stable, recovering within 90% of the recovered values on date of manufacture for on-going stability studies. **Conclusion:** VALIDATE® Body Fluids is a liquid-stable, ready-to-use product stored at freezer temperatures. The product is fit-for-purpose as a calibration verification test kit that covers the full AMR for 12 analytes commonly requested for body fluid analysis and the product conforms to CLSI EP06-A guidelines for linearity testing. The VALIDATE® Body Fluids supports laboratorians' method validation for assays used for body fluid specimens. The Roche cobas® 6000 formulation is currently available and listed with the FDA.

B-376

The New Dibucaine CHE (LN 4S21) Assay is Liquid and Ready-to-use

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OBJECTIVE: To present performance and interference test results of the new Abbott ARCHITECT Dibucaine CHE assay on the *c*System instrument. **RELEVANCE:** The new Dibucaine CHE assay (DIBCH, list number [LN] 4S21) is liquid, ready-to-use, and measures cholinesterase activity in plasma and serum in the presence of dibucaine (a cholinesterase inhibitor). This assay should be used, in combination with the Cholinesterase assay (ChE, LN 6K92), to determine if a patient is at risk for prolonged apnea and paralysis after administration of succinylcholine- or mivacurium-based general anesthesia. **METHODOLOGY:** The new DIBCH assay method utilizes butyrylthiocholine inhibition at 37°C. Cholinesterase metabolizes butyrylthiocholine to thiocholine and butyrate. Thiocholine reduces hexacyanoferrate (III), detectable by its absorbance at λ =404nm, to hexacyanoferrate (II). Dibucaine inhibits butyrylthiocholine metabolism (to thiocholine and butyrate) by cholinesterase. With low thiocholine concentrations, minimal reduction of hexacyanoferrate (III) to hexacyanoferrate (II) occurs and minimal change in absorbance at λ =404nm is observed. A patient's Dibucaine Number (DN) is calculated using DIBCH and ChE assays results (*DN = 100 * [1 – DIBCH_assay_result / ChE_assay_result]*). A patient's DN correlates with their plasma cholinesterase phenotype and speed of recovery from succinylcholine- or mivacurium-based general anesthesia. **VALIDATION:** Table 1 displays performance characteristics of the new DIBCH assay (LN 4S21) relative to the predicate (LN 6K92). The highest acceptable interferent levels for the new DIBCH assay, for low (391-516 U/L) and high (2162-2718 U/L) analyte concentrations respectively are: 7.6 and 64.7 mg/dL conjugated bilirubin, 7.4 and 57.3 mg/dL unconjugated bilirubin, 250 and 2000 mg/dL hemoglobin, 602 and 1205 mg/dL human triglycerides, 62 and 250 mg/dL intralipid, 13.0 and 14.0 g/L protein. **CONCLUSIONS:** The new DIBCH assay (LN 4S21) is liquid and ready-to-use. Like the predicate, it is used in combination with the ChE assay (LN 6K92) to determine a patient's DN.

Table 1. Comparison of performance characteristics of the new Dibucaine CHE assay (LN 4S21) and predicate (LN 6K92) and interference results for the new Dibucaine CHE assay (LN 4S21).

Characteristic	New Assay				Predicate Assay			
Configuration	Liquid, Ready-to-Use				Lyophilized			
Sample Type	Serum and plasma				Serum and plasma			
Sample Volume	4.0 µL				4.0 µL			
Measuring Interval (MI)	Analytical (AMI): 83 – 4000 U/L Extended (EMI): > 4000 – 16000 U/L				44 – 16000 U/L			
Verified Auto-dilution Application	1:4				none			
Imprecision	N	Mean (U/L)	Within-lab SD (U/L)*	Within-lab %CV*	N	Mean (U/L)	Within-lab SD (U/L)*	Within-lab %CV*
	84	109	14	12.9	48	938	54.3	5.8
	84	495	25	5.0	48	1573	83.0	5.3
	84	1244	18	1.5	48	1915	92.2	4.8
	84	1332	28	2.1	-	-	-	-
	84	1971	25	1.3	-	-	-	-
	84	3574	46	1.3	-	-	-	-
Stability: On-Board Calibration	28 days 168 hours				42 days 720 hours			

Study		New (y) vs. Predicate (x)	Abbott ARCHITECT Intraplatform *c*System**
Method Comparison – Dibucaine CHE Assay Result	N	130	127
	R	0.998	0.998
	Equation	y = 1.02x + 30.79	y = 0.97x + 33.68
	Range (U/L)	89 – 3949	99 – 3710
Method Comparison – Dibucaine Number	N	130	-
	R	0.986	-
	Equation	Y = 1.01x – 1.85	-
	Range (U/L)	70 - 91	-

* Per CLSI EP05-A2, the term *total-precision* was replaced with *within-laboratory (within-lab) precision.*
**Intraplatform comparison (ARCHITECT *c*16000 vs. *c*8000) for Dibucaine CHE (LN 4S21).

B-377

Development of a biochip array for the rapid, simultaneous detection of Pepsinogen I, Pepsinogen II and Gastrin 17, on the new random access, fully automated Evidence Evolution analyser

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Background: Atrophic gastritis (AG) is associated with a significantly higher risk of developing gastric cancer; the fifth most common cancer worldwide, in addition to enhancing the risk of malabsorption of vitamin B_{12}, iron, magnesium and zinc. AG involves a loss of 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 respectively. During atrophic corpus gastritis, the levels of PGI in circulation are decreased and the ratio of PGI:PGII is also lowered. G17 is a crucial peptide hormone of the gastrointestinal tract and is secreted by the G cells in the antrum. During antral atrophy the levels of G17 are ultimately decreased. These three biomarkers are therefore valuable in the screening of AG and can provide a comprehensive diagnosis on the condition of the gastric mucosa. Individual enzyme-linked immunosorbent assays (ELISAs) have been developed for the single detection of PGI, PGII and G17 in plasma (Biohit Oyj, Helsinki, Finland). The objective of this study is to utilize Randox's patented Biochip Array Technology (BAT) to develop a multiplex product, which enables the simultaneous detection of PGI, PGII and G17 in a single plasma sample. **Methods:** Simultaneous chemiluminescent sandwich immunoassays were employed, with analyte-specific capture antibodies immobilised on the biochip surface. The immunoassay was applied to the Evidence Evolution analyser, Randox's newest, high throughput analyser. The Evidence Evolution can produce the first set of results in 36 minutes, and one set of results per minute thereafter, enabling rapid sample screening. Functional and analytical sensitivity were assessed to confirm assay performance characteristics. Assay performance was further evaluated through precision and cross reactivity assessments in accordance with Clinical and Laboratory Standards Institution (CLSI) guidelines. **Results:** Nine-point calibration curves for each individual analyte were simultaneously generated with assay ranges 0-300ng/mL for PGI, 0-50ng/mL for PGII and 0-40pmol/L for G17. Functional sensitivity was recorded as 4.26ng/mL for PGI; 0.56ng/ mL for PGII and 0.42pmol/L for G17. Total assay precision (%CV) was determined as 8.2%-10.1% for PGI, 5.9% - 6.6% for PGII and 9.3% - 10.0% for G17. Cross reactivity testing demonstrated that each individual assay was specific for its target analyte (<1% cross reactivity with the other analytes). The array demonstrated no interference with common interferents tested (haemoglobin, bilirubin, triglycerides and intralipids). Good agreement was observed in correlation studies between the new, fully automated analyser system and individual reference ELISAs. **Conclusion:** The results of this collaborative study indicate applicability of the Evidence Evolution for the rapid and simultaneous measurement of PGI, PGII and G17 from a single plasma sample. The use of this biochip array facilitates rapid screening and diagnosis of patients at risk of developing gastric cancer

with a time to first result of 36 minutes. This offers advantages over current methods such as gastroscopy, which can be highly invasive, time consuming and costly.

B-378

Comparison of vitamin D assays ability to detect 25-hydroxyvitamin D in healthy volunteers, dialysis patients, and subjects taking vitamin D2 supplements

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Background: 25-hydroxyvitamin D (25(OH)D) testing and subsequent assay development has rapidly increased in recent years due to increased awareness of the clinical consequences of vitamin D deficiency. Accurate determination of 25(OH)D has proven to be difficult due to the tight association of 25(OH)D to vitamin D binding protein, unequal measurement of 25(OH)D2 and 25(OH)D3, and cross-reactivity of assays to 25(OH)D3 epimers. In this study, we compared the Fujirebio and Abbott 25-hydroxyvitamin D immunoassays to a VDSCP certified LC-MS/MS method. **Methods:** Serum samples from 50 healthy African American (n=25) and Caucasian (n=25) volunteers, 50 African American hemodialyzed patients, and 236 subjects taking varying concentrations of vitamin D2 supplements were assayed for 25-hydroxyvitamin D. Passing-Bablok and Bland-Altman analyses were used to determine Lumipulse and Architect assay correlation to the LC- MS/MS reference method. Agreement between the two immunoassays and the LC-MS/MS method was evaluated by calculating the concordance correlation coefficient (CCC). **Results:** The overall CCC between the two assays and the LC-MS/MS method were 0.6419 (Fujirebio) and 0.465 (Abbott). The CCC ranged from 0.936 (healthy volunteers) to 0.458 (vitamin D2 subjects) for the Abbott assay and from 0.919 (hemodialysis patients) to 0.586 (D2 subjects) for the Fujirebio assay. (See Figure) The overall mean bias (SD) for the two assays were -12.31 (12.5) and -13.51 (16.39) for the Fujirebio and Abbott assays, respectively. The mean bias ranged from -17.57 (17.25) for the vitamin D2 group to 0.56 (3.79) for healthy volunteers when assayed on the Abbott instrument and from -15.59 (13.39) to -4.04 (3.01) for these same groups using the Fujirebio assay. **Conclusions:** Correlation between the two immunoassays and the LC-MS/MS method was poor for the vitamin D2 supplementation group and overall, the Fujirebio and Abbott assays under-recovered 25(OH)D when compared to the LC-MS/MS reference method in all groups.

Table1: Mean 25-hydroxyvitamin D values, Passing Bablok regression and concordance correlation analysis of Fujirebio and Abbott immunoassays against an LC-MS/MS reference method for healthy volunteers, hemodialysis patients, and subjects taking varying concentrations of vitamin D2 supplements.

Population	Method	Mean 25-hydroxyvitamin D values (ng/ml)			Passing Bablok regression		Concordance correlation		
		Mean (95% CI)	*SD*	*Range*	*Slope (95% CI)*	*Intercept (95% CI)*	*CCC (95% CI)*	*r*	*Cb*
Overall N=336	Fujirebio	24.9 (23.4-26.4)	13.8	4-114.1	0.64 (0.588-0.687)	2.20 (1.003-3.125)	0.642 (0.598-0.682)	0.879	0.730
	Abbott	23.7 (22.4-25.0)	12.0	<3.4-119.6	0.52 (0.463-0.588)	4.66 (2.961-5.981)	0.465 (0.412-0.516)	0.721	0.645
	LC-MS/MS	37.2 (34.8-39.6)	22.7	<4-163.4	*Reference method*				
Healthy Volunteers N=50	Fujirebio	20.5 (18.7-22.3)	8.8	5.6-39.2	0.88 (0.777-0.949)	-0.39 (-2.439-0.956)	0.867 (0.798-0.914)	0.958	0.905
	Abbott	25.1 (26.6-29.6)	11.2	7.7-48.3	0.92 (0.805-1.036)	1.38 (-1.043-4.146)	0.936 (0.893-0.963)	0.942	0.994
	LC-MS/MS	24.5 (21.4-27.6)	10.2	7.0-52.0	*Reference method*				
Hemodialysis Patients N=50	Fujirebio	24.9 (22.5-27.3)	15.1	4-89.3	0.78 (0.748-0.816)	1.47 (0.1845-2.642)	0.919 (0.883-0.944)	0.990	0.928
	Abbott	21.8 (18.7-24.9)	13.5	<3.4-91.4	0.60 (0.519-0.715)	4.45 (1.797-5.667)	0.760 (0.656-0.835)	0.909	0.836
	LC-MS/MS	30.1 (27.3-32.9)	19.3	<4-111.8	*Reference method*				
Vitamin D2 Supplementation N=236	Fujirebio	25.8 (21.6-30)	14.3	4-114.1	0.60 (0.538-0.652)	1.34 (-0.3988- 3.18)	0.586 (0.532-0.636)	0.875	0.670
	Abbott	23.9 (20.1-27.7)	11.7	<3.4-119.6	0.47 (0.410-0.546)	4.37 (2.427-6.006)	0.406 (0.346-0.462)	0.737	0.551
	LC-MS/MS	41.4 (35.7-46.5)	24.0	<4-163.4	*Reference method*				

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Performance Evaluation of the Atellica CH AAT, C3, C4, CRP_2 and Hapt Assays

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Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH AAT, C3, C4, CRP_2, and Hapt Assays on the Atellica CH Analyzer. Measurement of AAT is used in diagnosing juvenile and adult cirrhosis of the liver. Measurements of C3 and C4 are used in determining inherited or acquired diseases, as well as diagnosing inflammatory and necrotic disorders. Measurement of CRP_2 is used in evaluating infection, tissue injury, and inflammatory diseases. Measurement of Hapt is used to aid in evaluating hemolytic disorders. The AAT, C3, C4, CRP_2 and Hapt assays all use antibody reactions that increase turbidity. The turbidity is proportional to the amount of analyte in the sample. **Method:** Performance testing included precision and accuracy. Assay precision was evaluated using the 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 patient sample results compared to results from the ADVIA® 1800 Clinical Chemistry System. **Results:** For AAT, within-lab precision ranged from 1.8-2.7% CV in serum samples. For C3, within-lab precision ranged from 1.4-2.0% CV in serum samples. For C4, within-lab precision ranged from 1.3-1.8% CV in serum samples. For CRP_2, within-lab precision ranged from 0.8-2.3% CV in serum/plasma samples. For Hapt, within-lab precision ranged from 2.2-2.9% CV in serum samples. The AAT serum method comparison study yielded a regression equation of y = 0.99x 4 mg/dL with r = 0.994, versus the ADVIA Chemistry 1800 AAT Assay. The C3 serum method comparison study yielded a regression equation of y = 0.99x + 0.6 mg/dL with r = 0.999, versus the ADVIA Chemistry 1800 C3 Assay. The C4 serum method comparison study yielded a regression equation of y = 0.96x + 0.4 mg/dL with r = 0.999, versus the ADVIA Chemistry 1800 C4 Assay. The CRP_2 serum method comparison study yielded a regression equation of y = 0.95x + 0.0 mg/dL with r = 0.989, versus the ADVIA Chemistry 1800 CRP_2 Assay. The Hapt serum method comparison study yielded a regression equation of y = 1.06x + 0 mg/dL with r = 0.997, versus the ADVIA Chemistry 1800 HAPT Assay. **Conclusions:** The Atellica CH AAT, C3, C4, CRP_2, and Hapt Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

B-380

Cardiac troponin T degradation - is blood matrix of importance?

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Background: Cardiac troponin T (cTnT) is a preferred cardiac biomarker for acute myocardial infarction (AMI) diagnosis. Recent studies demonstrated immunoreactive cTnT-derived degradation products in multiple pathologies (1-3). However, the cause of cTnT degradation was allocated to a pre-analytical effect caused by serum production (4-6). In this study, we investigated pre-analytical and *in vivo* cTnT degradation in multiple blood matrices and examined its impact on high-sensitivity cTnT (hs-cTnT) immunoassay results. **Methods**: The pre-analytical blood matrix influence on cTnT fragmentation was studied by adding intact cTnT to different blood tubes prior to blood withdrawal from a healthy volunteer. Subsequently, cTnT fragmentation was studied by immunoblotting. In addition, cTnT fragmentation was also investigated in simultaneously collected residual routine blood samples from patients with AMI. cTnT concentrations on residual routine blood samples collected at identical time points were determined by the hs-cTnT immunoassay. **Results**: When supplementing intact cTnT (40 kDa) in blood matrices of interest prior to blood withdrawal, it became apparent that cTnT is immediately and completely degraded to its primary fragment (29 kDa) in serum. Only minor cTnT degradation was observed in EDTA-, citrate- and hirudin-plasma. Li-heparin also showed minor cTnT fragmentation, though a thus far unseen cTnT fragment (26 kDa) was formed in this matrix. Residual blood samples of patients with AMI showed identical results. hs-cTnT immunoassay results on residual routine blood samples collected at identical time points (n=68) revealed no median significant difference between manufacturer approved blood matrices (p>0.05), though clinically significant individual differences were observed. **Conclusion**: This study revealed that cTnT degradation occurs *in vivo* and due to pre-analytical influence that significantly differs between peripheral blood matrices and impacts hs-cTnT immunoassay results at an individual level.

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B-381

Abbott Alinity c System Sigma Metrics for Clinical Chemistry Assays

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Background: Assay performance is dependent on the accuracy and precision of a given method. These two attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for more than 17 clinical chemistry assays tested on the Alinity c system. In 2017, a separate and distinct subset of 40 CC assays were analyzed and presented in two AACC posters using similar methods of analysis. **Methods:** A sigma metric was estimated for each assay and was plotted on a method decision chart. The sigma metric was calculated using the equation: *sigma = (%TEa - |%bias|) / %CV*. A precision study was conducted at Abbott on each assay using the Alinity c system per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, >100 samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity c and ARCHITECT *c*8000 systems. The 1st replicate from the Alinity c system was regressed versus the mean ARCHITECT *c*8000 concentration and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated near a critical concentration level. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity c system and the ARCHITECT *c*8000 system, where the ARCHITECT *c* system within-laboratory %CV and mean concentration values were obtained from the assay package insert. **Results:** The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near a critical concentration level. The precision profile charts of the within-laboratory %CV results for the Alinity c system overlaid with the ARCHITECT *c* system showed similar performance across the subset of assays evaluated. **Conclusion:** Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity c system assays had sigma metrics greater than 5. The precision performance on the Alinity c and ARCHITECT *c* systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

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Performance of the Sentinel Diagnostics C-Reactive Protein Ultra (MP)® Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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Introduction: The Sentinel Diagnostics C-Reactive Protein (CRP) Ultra (MP) is an immunoturbidimetric assay which quantitatively determines the concentration of C-reactive protein in serum or plasma. C-reactive protein is an acute phase protein synthesized in the liver in response to proinflammatory cytokines. CRP binding to ligands exposed during cell death and CRP binding to bacterial surfaces activates the complement cascade and stimulates phagocytosis. Therefore, CRP levels increase in infection, inflammation, tissue infarction, and trauma. CRP measurement may also be used for monitoring response to treatment and screening for infection postoperatively. **Method:** The CRP Ultra MP Assay reagent contains latex particles with adsorbed anti-CRP polyclonal antibody. The antibody binds to CRP in the patient sample resulting in agglutination. The increased turbidity in the reaction solution is detected as an absorbance change at 575nm. The rate of change in absorbance is directly proportional to the concentration of CRP in the sample. The assay is conducted using 2.0 uL of patient sample and the two CRP Ultra reagents. Two-point rate is calculated and converted to a concentration using a cubic spline calibration model. **Results:** The performance of the CRP Ultra (MP) Assay was assessed on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System. We evaluated accuracy with 95 serum samples (0.057 – 33.710 mg/dL) on the VITROS 4600 and VITROS 5600 Systems compared to the VITROS® 5,1 FS Chemistry System. The VITROS 4600 and VITROS 5600 Systems showed excellent correlation with the VITROS 5,1 FS System. VITROS 4600 = 0.99 * VITROS 5,1 FS + 0.07; (r) = 0.995. VITROS 5600 = 1.03 * VITROS 5,1 FS + 0.09; (r) = 0.995. A 22-day precision study conducted on the VITROS 4600 and VITROS 5600 Systems demonstrated optimal precision: CRP patient pools targeted at ≤ 0.5 mg/dL, ~0.5 mg/dL and > 0.5mg/dL resulted in within-laboratory percent coefficient of variation (%CV) of 2.55%, 2.44% and 1.77% respectively, for the VITROS 4600 System and 2.47%, 1.79% and 2.03% respectively, for the VITROS 5600 System. Linearity was evaluated using a 14 level admixture series. The observed linear range for the VITROS 4600 and VITROS 5600 Systems was 0.494 – 33.869 mg/dL and 0.475 – 34.332 mg/dL, respectively. The Limit of Quantitation (LoQ) for the VITROS 4600 and VITROS 5600 Systems was 0.024 mg/dL and 0.023 mg/dL, respectively based on 120 determinations with 10 low-level CRP serum samples. The Limit of Detection (LoD) for the VITROS 4600 and VITROS 5600 Systems was 0.027 mg/dL and 0.026 mg/dL, respectively based on 120 determinations with 2 CRP serum samples. The Limit of Blank (LoB) for the VITROS 4600 and VITROS 5600 Systems was 0.017 mg/dL and 0.014 mg/dL, respectively based on 120 determinations with 2 blank samples. **Conclusions:** The CRP Ultra (MP) assay evaluated on the VITROS 4600 and VITROS 5600 Systems exhibited excellent correlation with VITROS 5,1 FS System, optimal precision, linearity and low end sensitivity.

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High Levels of Serum Lipase without evidence of Pancreatitis in Emergency Room patients: case reports.

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Background: Several illnesses other than pancreatitis can result in increased serum lipase levels, such as reduction of renal clearance, neoplasia, critical illness, non-inflammatory pancreatic disease, diabetes, drugs and infections. Elevation of lipase is also described in patients with acute gastroenteritis, especially younger patient but it is not considered as a marker of poor prognosis. The objective of this study was to describe three case-reports of patients seen in the Emergency Room with increased serum levels of lipase in the absence of pancreatitis. **Methods:** Case reports, with retrospective analysis of medical records. **Results:** CASE 1. A 17-year old male presented with recurrent vomiting and epigastric abdominal pain. Abdomen was tender on palpation. Serum lipase was 1090 U/L. Abdominal ultrasonography was normal. Abdominal CT showed bowel distension and normal pancreas. Four hours later, serum lipase had dropped to 347 U/L. The patient was febrile and developed vomiting, requiring hospitalization. The next day, lipase levels decreased to 117 U/L and the patient was discharged uneventfully. CASE 2. A 37-year old woman presented with a 36-hour history of persistent nausea, with no relief on antiemetic medications. The physical examination was normal. Serum lipase was 1263 U/L. A CT scan of abdomen showed no pathological changes. Patient remained under observation, with intravenous hydration and symptomatic medications. On the morning of the following day, lipase dropped to 421 U/L, and the patient was discharged shortly thereafter. CASE 3. A 19-year old man presented with a 48 hour-history of vomiting and diarrhea. The abdomen was diffusely painful, with no signs of peritoneal irritation. Abdominal ultrasound and CT scan were normal. Serum lipase was 1329 U/L. The patient was admitted to the hospital for intravenous hydration and symptomatic medication. The next day, serum lipase dropped to 143 U/L. Patient was discharged uneventfully shortly thereafter. The same laboratory methodology was used in all cases, with reference values for lipase = 73 to 393 U/L. In all three cases, a rapid decline in serum lipase levels was seen. Serum amylase levels remained normal at all times. In all three cases, all the laboratory results were confirmed with another assay and documented consistent results. **Conclusion:** We identified three cases of elevation of lipase without evidence of pancreatitis, indicating the existence of other causes for such elevation. The elevated lipase levels were transitory and did not seem to influence evolution or outcome. The differential diagnosis with pancreatitis continues to be relevant for decision analysis in an emergency room environment.

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Development of an Assay for Measurement of β2-Microglobulin (B2MG) in Urine Samples on Roche Clinical Chemistry Analyzers

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Background:
B2MG is a low-molecular-weight protein with approximately 12 kDa. It occurs on the cell-membrane of all nucleus-containing cells except trophoblasts. Due to its low molecular weight, it is rapidly filtered through the renal glomeruli. Thereafter, up to 99.9 % is reabsorbed by the proximal tubules.Acute changes in tubular reabsorption and progressive renal diseases causing irreversible structural tubular defects impair tubular reabsorption of numerous smaller proteins including B2MG. Thus, urinary B2MG is discussed as a marker for the diagnosis and monitoring of tubulointerstitial renal damage. Elevated B2MG levels may identify patients at higher risk of glomerular filtration rate (GFR) decline in other kidney diseases such as membranous nephropathy. Furthermore, there is evidence that B2MG excretion is associated with acute allograft rejection in renal transplant recipients.
Methods:
Immunoturbidimetric assay.Latex-bound anti-β2-microglobulin antibodies react with antigen from the sample to form antigen/antibody complexes which are determined turbidimetrically after agglutination.
Development Goals:
-Development of an application for analysis of urine samples based on the existing Tina-quant® β2-Microglobulin assay
-Measuring range: 0.2-5.8 mg/L (16.9-491.3 nmol/L)
-In use time β2-Microglobulin reagent: 12 weeks (cobas c pack and cobas c pack large)
-Low sensitivity to drug interference-Improved in use time for β2-Microglobulin calibrator and controls
Results:
The linear assay range of the B2MGU assay is 0.2-5.8 mg/L (16.9-491.3 nmol/L). Extended measuring range: Extended measuring range: 63.8 mg/L (5404.3 nmol/L). Limit of Blank: 0.1 mg/L (8.5 nmol/L), Limit of Detection: 0.15 mg/L (12.7 nmol/L), Limit of Quantitation: 0.2 mg/L (16.9 nmol/L) with 20% total error. B2MGU assay has been standardized against the WHO standard 1st WHO standard 1985, NIBSC code B2M). No interference of Albumin (5000 mg/L), Calcium 12.0 (mmol/L), Creatinine (10.0 mg/ml), Glucose (70.0 mg/ml) and Hemolysis (1100 mg/dL) was observed (Acceptance criteria: Recovery of β2-microglobulin concentrations ≤ 1.0 mg/L: ≤ ± 0.1 mg/L and > 1.0 mg/L: ≤ ± 10 % of initial value). Precision - CLSI EP5 - 21 days (Repeatability): SD ranging from 2.54 nmol/L (0.03 mg/L) to (0.13 mg/L), CV ranging from 2.3% to 9.4%, sample concentration range: 25.4 nmol/L (0.3 mg/L) to Precision - CLSI EP5 - 21 days (Repeatability): SD ranging from 0.09 mg/L to 0.25 mg/L, CV ranging from 0.7% to 2.3%, sample concentration range: 25.41 nmol/L (0.3 mg/L) to 465.85 nmol/L (5.5 mg/L). Human urine samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche cobas c 501 analyzer (x) (n = 118). Passing/Bablok regression: y = 0.967x - 0.0005 mg/L (0.04 nmol/L). The sample concentrations were between 0.21 and 5.76 mg/L (17.79 and 487.87 nmol/L). Comparison to B2MG urine method on Siemens ProSpec: y = 0.989x - 0.0539 mg/L (4.57 nmol/L).
Conclusion:
All of the development goals for the urine application of Roche Tina-quant® β2-Microglobulin assay were met. The introduction of the B2MG urine assay will complete the assay portfolio for screening and monitoring patients with kidney disease.

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Laboratory workflow analysis in special protein testing

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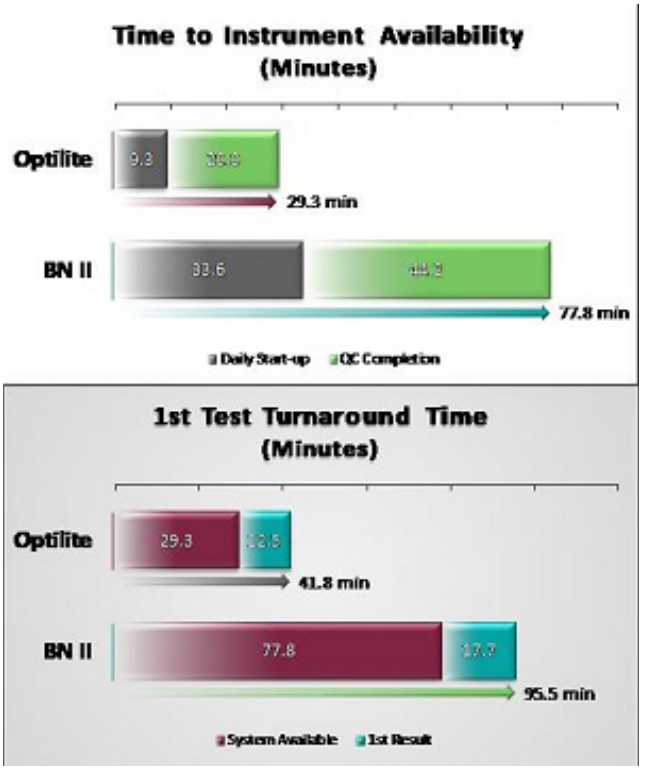
Background: Dedicated special protein analyzers play an important role in the clinical laboratory. Historically the Siemens BN™II system has been one of the most commonly utilized protein analyzers. The Binding Site Optilite® system is a new protein analyzer that has been recently introduced to the clinical laboratory market. The objective of this study was to compare workflow and time requirements for daily startup and test performance for both systems in an actual clinical diagnostic laboratory setting. **Methods**: A before and after time and motion study was performed by observing the daily start-up and testing activities for both systems. Observation data was collected for four days on each system with no variation in location, layout, workflow, scheduling and staffing. The special protein test menus were identical and average daily volumes were very similar. Testing was performed daily through two shifts which required a daily startup and shut down. The data is presented as weighted averages. **Results**: On average, the Optilite required 9.3 minutes to perform daily start-up while the BNII needed 33.6 minutes. Additionally, the Optilite required 20.0 minutes for daily QC, compared to 44.2 minutes for the BNII. Cumulative time to instrument availability was 29.3 minutes for the Optilite and 77.8 minutes for the BNII. The average time to first result on the Optilite was 12.5 minutes, compared to 17.7 minutes for the BNII. Total time to first result was 41.8 minutes for the Optilite and 95.5 minutes for BNII. **Conclusions**: The Optilite required significantly less time for start-up. This allows testing to begin sooner and contribute more staff time availability to perform other clinical testing. The Optilite special protein analyzer provides rapid start-up times, reduced QC times and improved result reporting which has a substantial impact on laboratory workflow, technician time and reporting speed.



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Development of a multiplex biochip array for early detection of Chronic Kidney Disease on the new fully automated Evidence Evolution analyser

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Background: Chronic Kidney Disease (CKD) defines the progressive loss of kidney function, over a period of time. The early stages of CKD progress asymptomatically, and are therefore difficult to diagnose. However, late diagnosis of CKD can ultimately lead to end-stage renal disease requiring kidney dialysis or transplantation. The use of a screening test for the detection of early stage CKD biomarkers has the potential to identify individuals at risk of developing progressive renal disease. Biochip array technology (BAT) facilitates the detection of multiple analytes from a single sample, allowing comprehensive sample screening. The objective of this study is to develop a multiplex biochip array for the simultaneous measurement of Fatty Acid-Binding Protein 1 (FABP1), soluble Tumour Necrosis Factor Receptors 1 and 2 (sTNF-R1 and sTNF-R2) and Macrophage Inflammatory Protein 1 alpha (MIP-1α), with utility for the early detection of CKD on a fully automated platform. **Methods:** Simultaneous

chemiluminescent sandwich immunoassays were employed. Capture antibodies were immobilised on discrete test regions on the biochip surface and applied to the fully automated Evidence Evolution biochip analyser. Sensitivity, repeatability, within lab precision, assay specificity and interference were evaluated in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, in order to confirm assay performance characteristics. A comparative study was conducted with plasma patient samples (n=40) from controls and defined CKD stages, classified by estimated Glomerular Filtration Rate (eGFR) using the CKD Epidemiology Collaboration (CKD-EPI) equation. **Results:** Each analyte was tested simultaneously and utilised assay ranges of 0-200ng/mL for FABP1; 0-10ng/mL for sTNF-R1; 0-130pg/mL for MIP-1α and 0-20ng/mL for sTNF-R2. Assay sensitivity was recorded as 1.56ng/mL for FABP1; 0.04ng/mL for sTNF-R1; 0.50pg/mL for MIP-1α and 0.16ng/mL for sTNF-R2. Average repeatability (%CV) was determined to be 9.62% for FABP1, 8.78% for sTNF-R1, 8.07% for MIP-1α and 10.61% for sTNF-R2. Average within-lab precision was determined to be 16.75% for FABP1, 11.13% for sTNF-R1, 10.74% for MIP-1α and 15.93% for sTNF-R2. Cross reactivity analysis determined that each individual assay was specific for its target analyte. Additionally, no cross reactivity was observed with non-panel, homologous proteins (cross reactivity <1%). The assay demonstrated no significant interference with common interferents tested (triglycerides, haemoglobin, intralipids and bilirubin). A cohort of 40 samples assessing normal plasma samples compared to Stage 1, 2 and 3 CKD plasma samples (n = 10 each), yielded AUC values for individual biomarkers in the range of 0.705-0.905, differentiating normal controls from early CKD stages. **Conclusions:** The findings of this study highlight the utility of a multiplex immunoassay array for the early detection of CKD, through rapid, fully-automated, simultaneous measurement of FABP1, sTNF-R1, sTNF-R2 and MIP-1α on the Evidence Evolution analyser platform. This biochip array provides a valuable and reliable multi-analytical tool for the identification of early CKD.

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Development of a biochip array for the detection of Adhesion Molecules on the new random access fully automated Evidence Evolution analyser

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Background: Cell adhesion molecules are complex membrane proteins which mediate cell-to-cell interactions and subsequently influence a wide range of intracellular signalling cascades. Adhesion molecules can also be detected in soluble forms in the circulation. These molecules are implicated in a diverse range of physiological processes such as cell proliferation, migration, differentiation, apoptosis, and the mediation of inflammatory processes. Altered circulating levels of adhesion molecules have been reported in a wide range of physiological conditions, such as cardiovascular disease, stroke, cancer, chronic kidney disease (CKD) and diabetes. Consequently, the measurement of circulating adhesion molecules has importance for identifying and monitoring disease. The objective of this study is to utilize Randox's proprietary, multiplexing biochip array technology to develop an Adhesion Molecule array. The array, encompassing five adhesion molecules - Vascular Cell Adhesion Molecule 1 (VCAM-1), Intracellular Adhesion Molecule 1 (ICAM-1), E-selectin (ESEL), P-selectin (PSEL) and L-selectin (LSEL) - was applied to the new, fully-automated Randox Evolution Analyser. **Methods:** Antibodies specific to VCAM-1, ICAM-1, ESEL, PSEL and LSEL were immobilised to discrete testing regions within a biochip surface and a chemiluminescent sandwich immunoassay format was used for this array. The array has been developed on the Evidence Evolution analyser which requires minimal user input and provides rapid results, with the first test read after 36 minutes and a test per minute thereafter. Assay sensitivity, precision, cross reactivity and interference were evaluated to define assay characteristics. Clinical utility was also evaluated using a cohort of 41 samples (22 healthy controls and 19 CKD). **Results:** The assays were simultaneously evaluated and yielded the following ranges and sensitivities - VCAM-1, range 0-6600 ng/ml and sensitivity 34ng/ml; ICAM-1, range 0-2000 ng/ml and sensitivity 13ng/ml; ESEL, range 0-500 ng/ml and sensitivity 4.0ng/ml; PSEL, range 0-2400 ng/ml and sensitivity 29ng/ml, and LSEL, range 0-7000 ng/ml and sensitivity 36ng/ml. Average repeatability was recorded as 4.5% for VCAM-1; 5.6% for ICAM-1; 12.8% for ESEL; 3.5% for PSEL and 5.7% for LSEL. Average within lab precision of 6.8% was observed for VCAM-1; 8.8% for ICAM-1; 16.6% for ESEL; 4.7% for PSEL and 8.2% for LSEL. Cross reactivity analysis demonstrated that each individual assay was specific for its target analyte (cross reactivity <1%) and that no cross reactivity was observed with non-panel homologous proteins (cross reactivity <1%). Common blood interferents - haemoglobin, triglycerides, intralipids and bilirubin - demonstrated no interference with assay performance. Significant differences in biomarker concentrations were observed when CKD samples were compared to controls - VCAM-1 (AUC = 0.706; p = 0.010), ICAM-1 (AUC = 0.789; p = 0.006) and LSEL (AUC = 0.610; p = 0.007). **Conclusion:** This study reports on the development of a multiplexed array for the simultaneous measurement of VCAM-1, ICAM-1, ESEL, PSEL and LSEL. This array offers a rapid, fully-automated alternative to traditional ELISA methods, with minimal sample volume requirements. This newly developed Adhesion Molecule array can be applied to a diverse range of pathologies.

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Development of a liquid Procalcitonin calibration verification set to verify the method's analytical range

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Introduction: Procalcitonin (PCT) is a 116 amino acid peptide precursor of the hormone calcitonin. It is a biomarker associated with the inflammatory response to bacterial infection. Localized infections, allergies, autoimmune diseases and transplant rejections do not usually induce a PCT response. PCT aids in the assessment of critically ill patients to determine if a patient has severe sepsis or is in septic shock. Healthy individuals will have a PCT of less than 0.1 ng/mL; a result above this can indicate a bacterial infection requiring treatment. If responsive to therapies, the PCT value will decrease and continue to decrease until reaching normal levels. PCT immunoassay methods have been cleared by the U.S. FDA for several automated platforms, including the bioMérieux VIDAS®, Roche Diagnostics cobas®, Abbott ARCHITECT and Beckman Coulter AU. As non-waived laboratory tests, calibration verification is required under CLIA '88. Our objective was to develop a liquid-stable, human serum based PCT calibration verification test kit for use by clinical laboratories' method validation. **Methods:** VALIDATE® Procalcitonin was formulated in a human-serum matrix according to CLSI EP06-A into five equal delta concentrations to cover the analytical measuring range (AMR), 0.02 to 100 ng/mL, of the Roche cobas® 6000's PCT reagent. In total, 5 individual lots were manufactured. For each level, samples were tested in triplicate on the Roche cobas® 6000 analyzer. Reported recoveries were evaluated for mean, SD and linearity using MSDRx® (LGC Maine Standards' proprietary linearity software). Limits were applied as 50% of the total allowable error (TAE) for the analyte. To establish product stability claims, samples were subjected to one freeze-thaw cycle, followed by a 24-hour, 22ºC stress condition and four subsequent open-vial events. Stability testing is on-going. **Results:** Procalcitonin is linear and within applied TAE limits through the validated reportable range of the Roche cobas® PCT assay. Linear regression analysis of theoretical vs recovered was Y = 1.007 X + 0.031, R^2 = 0.9984. Typical recovered values for level 1 and 5 are 0.08 ng/mL and 92 ng/mL respectively. All levels were stable, recovering within 90% of the recovered values on date of manufacture in on-going real time and stress stability studies. A comparison of the validated range against the Roche cobas® 6000 demonstrates full reportable range coverage for the analyte in a human-serum matrix. **Conclusion:** VALIDATE® Procalcitonin is a liquid-stable, ready-to-use product stored at freezer temperatures. The product is fit-for-purpose as a calibration verification test kit that covers the full AMR for the Roche cobas® 6000 PCT reagent. The product conforms to CLSI EP06-A guidelines for linearity testing. The Roche cobas® 6000 formulation is currently available and listed with the FDA. Formulations for the Abbott ARCHITECT, bioMérieux VIDAS® and Beckman Coulter AU are currently in development.

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Clinical utility of cystatin C as a biomarker of kidney function

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Background: Chronic kidney disease (CKD) is a public health problem. In Brazil, it is estimated that in 2014, 112,004 patients undergo dialysis as a consequence of hypertensive nephropathy and diabetes. Glomerular filtration rate (GFR) is the main indicator of renal function, correlating with the severity of CKD and reduced before the onset of clinical manifestations. Studies indicate that Cystatin C has a higher sensitivity than creatinine in detecting renal disease and in assessing risk for clinically relevant events such as heart failure, hypertension, and diabetes. Cystatin C is a non-glycosylated protein, produced at a constant rate and freely filtered by the kidneys, less influenced by variables such as age, sex, race, muscle mass and physical activity than serum creatinine and has the sensitivity of detecting small reductions in renal function in patients with GFR 60-90 mL/min/1.73m2, even with creatinine concentrations within normal range, providing high sensitivity and specificity in the evaluation of GFR. The KDIGO guideline, published in 2013, reports that the CKD-EPI creat/cyst equation that combines Cystatin C and serum creatinine has obtained better results in the estimation of GFR and classification of patients with renal disease. The purposes of this study were to evaluate the performance of Cystatin C against

serum creatinine when analyzed alone; the agreement between eGFR based on formulas derived from Cystatin C when compared with the equation derived from serum creatinine and the possible influence of some parameters on the magnitude of the differences between the different equations derived from Cystatin C and creatinine for eGFR and to analyze the reuptake of DRC based on these different equations. **Methods:** Population study with 164 adult patients at different stages of CKD. Serum and urinary creatinine analyzed by the enzyme method in Advia 2400 and cystatinC were analyzed in BN II by the immunonephelometric method. The CKD-EPI-derived cystatinC (CKD-EPI cyst and CKD-EPI creat/cyst) and creatinine-derived (CKD-EPI creat) equations were used to estimate GFR. **Results**: The study of correlation, trend and determination of cut-off value of DPU24h indicate that both cystatinC and serum creatinine results show the same results when we associate the isolated cystatin C and creatinine results to the DPU24h result. The results of creatinine and cystatin C begin to extrapolate the upper limit of the Reference Interval when the DPU24h presents values below the range of 79 to 80mL/min/1.73m2. The values of DPU24h lower than 79mL/min/1.73m2 show creatinine and cystatinC results above the upper limit of the reference range. The comparison study between the CystatinC-derived equations and the creatinine-derived equation showed that CKD-EPI creat/cyst is much more precise and accurate than CKD-EPI cyst. Proteinuria and albuminuria were statistically significant for the group of patients between stages 3b and 5 of CKD and 25% of patients presented a different staging when compared to CKD-EPI creat/cyst result with CKD-EPI creat. **Conclusions:** Isolated CystatinC and creatinine were more sensitive in detecting alterations in renal function, and the CKD-EPI creat/cyst equation, recommended by KDIGO in specific situations, presented excellent accuracy and precision when compared to CKD-EPIcreat, is recommended by Brazilian medical societies.

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Development of an enzymatic assay to measure lactate in perchloric acid-precipitated cerebrospinal fluid

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Background: Individuals with inherited deficiencies of the pyruvate dehydrogenase complex or the respiratory chain complex can have increased concentrations of cerebrospinal fluid (CSF) lactate. Such measurements are clinical useful when measured in conjunction with pyruvate in order to calculate the lactate:pyruvate (L:P) ratio, a useful surrogate of cytosolic redox status. CSF pyruvate is measured in a protein-free supernatant prepared by the addition of CSF to perchloric acid while lactate is measured in untreated CSF. Utilizing the same sample for both lactate and pyruvate measurements is desirable. The objective was to develop a method to measure lactate in perchloric-acid precipitated CSF and validate the L:P ratio as calculated from the analysis of both analytes in the same sample. **Methods:** Samples were prepared by the addition of 1 mL CSF to 2 mL 8% (w/v) cold perchloric acid, incubated on ice for 10 min, then centrifuged to obtain a protein-free supernatant. Lactate was measured by its oxidation to pyruvate and hydrogen peroxide using lactate oxidase and the absorbance of the resulting chromogen determined at 540 nm on a Roche cobas c501 chemistry analyzer. Method accuracy, linearity, imprecision and sensitivity were determined and a reference interval was verified. **Results:** To assess accuracy, this method was compared to lactate determined in unaltered CSF at another laboratory using 41 specimens with lactate concentrations from 0.6-11.9 mmol/L. Linear regression produced a slope of 1.09 and y-intercept of 0.26 (R^2 =1.00). Recovery was performed by ad-mixes of a high lactate standard and a CSF pool in different ratios to create a set of 19 samples prior to preparing protein-free supernatants. Recovery was 94.6-100% (mean±SD was 97.4±1.4%) at lactate concentrations of 2.68 to 12.63 mmol/L. Linearity was determined by combining two supernatants with low and high lactate concentrations in different ratios to create a set of six samples (0.15-12.70 mmol/L) that were tested in duplicate. Linear regression generated a slope of 1.01, y-intercept of -0.04 (R^2=1.00). Precision was verified by analyzing quality control materials (acid-treated lactate standard) in 3 replicates each day for 5 days. Within-laboratory imprecision was 2.3% at 1.5 mmol/L and 1.5% at 10.5 mmol/L. The limit of blank was 0.05 mmol/L as determined by the mean added to three standard deviations determined from 10 replicates of perchloric-acid treated saline pool. The limit of detection was determined to be 0.12 mmol/L calculated from 10 replicates of a patient sample treated with perchloric-acid. The manufacturer's reference interval of 1.1-2.4 mmol/L was verified using 20 residual patient CSF samples. **Conclusion:** CSF lactate can be measured with accuracy and precision using the same perchloric-acid treated sample that is used for pyruvate.

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Performance verification and assessment of the consistency of four Lp-PLA2 activity reagents

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Background: To validate the analytical performance and the consistency of four Lp-PLA2 activity reagents (Evermed, DiaSys, Hengxiao and Zhongyuan were labeled as A, B, C and D, respectively) on Beckman Au5800 automatic biochemical analyzer. **Methods:** Performance validation. The remaining serum samples of 214 patients and 140 apparently healthy individuals were collected during May to August 2017 in Peking Union Medical College Hospital (PUMCH) and used to method comparison and reference interval validation, respectively. According to the standard of CLSI EP15-A, EP6-A, EP-17and EP7-P,the precision, linearity range, sensitivity, common interference (free bilirubin, conjunct bilirubin , hemoglobin, chyle) were assessed. According to EP9-A2, method comparison was conducted and deviations of each reagent were compared in the medical decision level (328U/L, 391U/L, 485U/L). **Results:** The precision of four reagents were good and the repeatability *CV%* of A-D were 0.5%-1.7%, 0.7%-3.0%, 0.9%-2.0%, 0.5%-3.3%, and reproducibility *CV%* were 0.7%-2.9%, 1.4%-3.2%, 1.3%-1.9%, 0.8%-4.1%, Only B reagent is greater than the manufacturer's stated total *CV%*, but both are less than the 5% quality target in the laboratory .The linearity range of A to D were 44 -1992 U/L, 39 -1798 U/L, 13 -540 U/L and 75 -1717U/L, the regression coefficient R^2 was between 0.997 and 1.000, and the correlation coefficient (r) was between 0.998 and 1.000. The anti-interference of chyle were good among four reagents which met the manufacturer's claims or clinical needs. Bilirubin at low levels of Lp-PLA2 interference on C reagent obvious; B, C in the hemoglobin 4.5g / L was significantly negative interference, and D in hemoglobin 2.45g /L is showing interference. The regression coefficients R^2 of A, C, D compared with B were between 0.978 and 0.995, and the correlation coefficients (r) were between 0.989 and 0.998. The expected deviations at the medical decision level ranged from -240 U / L to 113 U / L. 131 (93.6%), 140 (100%), 82 (58.6%), and 128 (91.4%) of Lp-PLA2 activity test results were found to be within the manufacturer's stated reference intervals for A to D, respectively. **Conclusion:** The four Lp-PLA2 activity reagents, performed on automatic biochemistry analyzer, had good quality of precision and linearity range, but the anti-interference should be improved.

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Comparison of monoclonal protein concentration in serum measured by Hevylite or by electrophoresis

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Background: The measurement of the monoclonal protein (M-protein) is important for the diagnostic and follow-up of monoclonal gammopathies. The guidelines of clinical practices recommend that its concentration should be measured by serum protein electrophoresis (SPE) for the majority of cases. Immunochemical methods able to measure the isotype-specific heavy and light chain (HLC) are an alternative to measure the M-protein. Objective: Compare the HLC assay (Hevylite) with the electrophoretic methods of SPE, namely capillary electrophoresis (CE) and electrophoresis in agarose gel (AG), and estimate the systematic measurement error. **Methods:** The SPE was performed in a series of dilutions of 12 serum samples with M-protein (range 20-40 g/L, 6 isotypes with beta migration and 6 with gamma). Dilutions were performed with a pool of sera without M-protein and a pool of hipogammoglobulinemic sera. SPE was performed by CE (Capillarys 2 from Sebia) and AG (Hydrasys from Sebia). The integration of the M-protein peak in the electropherogram was performed by the perpendicular drop method. The measurement of the involved pair with the Hevylite assay was performed in the SPAplus analyzer (The Binding Site). The quantification methods for statistical comparison used were Pearson correlation, Passing-Bablok regression and Bland-Altman graphics. SPE was considered the reference method.

Results: For IgG M-proteins, Hevylite assay always retrieved a positive constant systematic error (SEc) ranging between 1.69 and 3.87 g/L, depending on the mobility and the SPE method. Hevylite showed proportional systematic error (SEp) only when it was compared to CE and M-protein migration was gamma. SEp underestimated the M-protein. For IgA M-proteins, Hevylite did not show SEc when comparing with AG. It showed SEc regarding CE, with a value ranging between -1.17 and -1.46, depending on the respective mobility. Hevylite did not show SEp when compared with the AG for M-protein IgA, except for beta migration patterns, and for CE when it has mobility gamma. SEp for all the other cases always overestimated the M-protein. For IgM M-proteins, Hevylite always presented SEc and SEp. SEc varied between -1.12 and -6.14 according to the mobility and the electrophoretic method used to compare. The SEp always overestimated the M-protein. **Conclusion:** Hevylite results show a better metrological comparability with the electrophoresis in agarose gel (although it presents a SEc < 3.23 g/L), especially for IgG and IgA M-proteins migrating in beta. Regarding capillarity electrophoresis, Hevylite is only comparable when the M-protein is IgG and it migrates in beta or is IgA migrating in gamma (SEc < 3.87 g/L, -0.94 g/L). Measurement results of IgM M-protein using Hevylite assay are not comparable with those from electrophoresis, since its SEp (slope >1.32) tends to overestimate the concentration. Nevertheless, Hevylite represents the advantage to be able to follow the uninvolved polyclonal pair, measuring the immunosuppression as a risk factor towards a shorter time to progression.

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The Performance of Diazyme PCT Assay on Beckman DxC 700 AU Analyzer

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Background: Procalcitonin (PCT) is a specific biomarker for systemic sepsis and septic shock. In healthy subjects, the PCT levels in circulation are very low (<0.05 ng/ml). Elevated circulating levels of PCT are important indicators in response to microbial infections. Diazyme PCT Assay (k162297) is a latex particle enhanced immunoturbidimetric method for the quantitative determination of PCT in human serum, EDTA or lithium heparin plasma. Measurement of PCT in conjunction with other laboratory findings and clinical assessments aids in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock. In this study, the performance of the PCT Assay was evaluated on the Beckman DxC 700 AU analyzer. Method: Diazyme PCT Assay is based on a latex enhanced immunoturbidimetric assay. It contains two reagents: reaction buffer and specific anti-PCT antibody coated latex particles. PCT antigen in the sample binds to the antibody coated latex particles and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of PCT in the sample. In this study, analytical performance of the assay was evaluated on the Beckman DxC 700 AU analyzer according to CLSI guidelines. Results*:* The precision of the Diazyme PCT Assay was evaluated according to modified CLSI EP5-A2 guideline. In the study, two levels of the PCT serum controls and two patient serum samples were tested in duplicate per run, 2 runs per day for 5 days. The within-run, between-run, between-day, and total CV% were ≤ 10% when PCT > 1.0 ng/mL and SD ≤ 0.1 ng/mL when PCT ≤ 1.0 ng/mL. Method comparison study was performed following CLSI EP9-A2 protocol. A total of 120 serum samples were compared on the Beckman DxC 700 AU and the master analyzer Beckman AU 400 and results yielded R^2 value of 0.9886 with a slope of 0.9529 and y intercept of -0.1113. Clinical sensitivity studies were conducted by testing 90 serum samples from patients on their first day of ICU admission. With cut-off at 0.5 ng/mL, the sensitivity, specificity and total agreement were 98.0%, 45.0% and 74.4%, respectively. With cut-off at 2.0 ng/mL, the sensitivity, specificity and total agreement were 92.0%, 77.5% and 85.6%, respectively. The assay was linear from 0.20 to 52.0 ng/mL with limit of quantitation (LOQ) of 0.20 ng/mL. Twenty interfering substances were tested and showed < ± 10% interference. Calibration was stable for 14 days, and reagents on-board were stable for 4 weeks. Conclusion: the performance of Diazyme PCT Assay on Beckman DxC 700 AU is accurate, sensitive and significantly equivalent to that on the master analyzer Beckman AU 400.

B-394

Performance Evaluation of the New ADVIA Chemistry Cystatin C_2 Assay*

P. Datta, P. Hickey, M. Chakrabarty, J. Dai. *Siemens Healthcare Diagnostics, Newark, DE*

Background: The ADVIA® Chemistry Cystatin C_2 assay* (CYSC_2 from Siemens Healthineers) is standardized to the IFCC international reference material, ERMDA-471. The assay measures the serum protein cystatin C in human serum or plasma and is useful in the diagnosis and treatment of renal insufficiency. Serum concentrations of cystatin C are almost totally dependent on the glomerular filtration rate (GFR) and not affected by factors that have been demonstrated to affect creatinine values such as muscle mass and nutrition. In addition, a rise in serum creatinine does not become evident until the GFR has fallen approximately by 50%. The ADVIA Chemistry Cystatin C_2 assay has been evaluated on the automated, random-access ADVIA® 1800, ADVIA® 2400, and ADVIA® Chemistry XPT Systems (Siemens). The evaluation of this assay included precision, linearity, interference, high dose hook, and method comparison studies. **Methods:** All ADVIA Chemistry Systems use the same CYSC_2 reagent packs, calibrators, and commercial controls. In this assay, a specimen containing human cystatin C is diluted and then reacted with antibody (rabbit) coupled to latex microparticles. The increased turbidity is measured at 571 nm. By constructing a six-level standard curve (water is used as reagent blank) from the absorbances of standards, the analyte concentration of the sample is determined. **Results:**
The repeatability and within-lab CVs (80 replicates per sample) of the new assay with three commercial controls (~0.5, 0.9, and 3.9 mg/L cystatin C) and two serum pools (1.1 and 8.8 mg/L cystatin C) on all ADVIA Chemistry Systems were <2.7% and <4.4%, respectively. The analytical range/linearity of the assay on all ADVIA Chemistry Systems was from 0.25 mg/L to the cystatin C concentration in the highest level of calibrator (8.96-9.65 mg/L). The assay on the ADVIA 1800 system (y) correlated well with the cystatin C assay on the BN ProSpec® System (x), also from Siemens (both assays are standardized to IFCC reference material): y = 1.01x + 0.10 (r = 0.99, n = 155, range: 0.56-6.93 mg/L). The ADVIA 2400 and ADVIA XPT CYSC_2 assays , in turn, agreed with the ADVIA 1800 CYSC_2 assay : ADVIA 2400 CYSC_2 = 1.00 (ADVIA 1800 CYSC_2) - 0.02 (r = 0.99, n = 155, range: 0.64-6.75 mg/L); and ADVIA XPT CYSC_2 = 1.01 (ADVIA 1800 CYSC_2) - 0.00 (r = 0.99, n = 155, range: 0.64-6.75 mg/L). The ADVIA CYSC_2 Assay showed <10% interference with bilirubin (conjugated or free) up to 60 mg/dL, with hemoglobin up to 1000 mg/dL, with lipids (INTRALIPID, Fresenius Kabi AB Corporation) up to 1000 mg/dL, and with rheumatoid factors up to 1200 IU/mL. The assay has a minimum of 60 days on-system and calibration stability on all ADVIA Chemistry Systems. No prozone was observed with the assay on any platform up to the highest cystatin C concentration tested in a sample (69.55 mg/L). **Conclusion:** We conclude that the Cystatin C_2 assay, standardized to ERMDA-471, when used on any ADVIA Chemistry System, can measure serum or plasma cystatin C concentrations precisely and accurately over a broad range in routine laboratory use.
*Currently under development.

Wednesday, August 1, 2018

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

TDM/Toxicology/DAU

B-395

Stability of ethanol in whole blood samples stored in a refrigerator

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Background: Determination of ethanol in whole blood samples is one of the most frequent analytical practices in the toxicology laboratory. This determination has very important legal consequences, such as traffic accidents, labor, maltreatment ... For all these reasons, it is necessary to maintain these samples properly, since they can be required at any time and should not suffer any variation in the concentration of ethanol. In this work, 40 samples of whole blood stored in a refrigerator at 4ºC will be studied and the stability of the ethanol will be checked. **Methods**: Ethanol concentration is analyzed in 40 samples of whole blood stored in the refrigerator, which were already analyzed in their day, each of them with a certain storage time. The results of the two measurements are compared and it is checked if the ethanol concentration is stable. If this is not the case, the possible causes of this variation are determined. For this study, a gas headspace chromatograph (HS-GC) is used, with a flame ionization as a detector (FID). Propanol is used as an internal standard to calculate the concentration of ethanol in the samples. **Results:** The results of the comparison show that:1. Samples that no contain ethanol obtained the same result, that is, no generation of alcohol occurs during storage.2. Samples that contain ethanol suffer a variation in their concentration, they all lose alcohol. This variation is due to several factors, including the storage time and the volume of the air chamber in the sample tubes. This loss is caused by the oxidation of ethanol to acetaldehyde, and later, to acetic acid. This oxidation, dependent on temperature, is catalyzed by oxyhemoglobin (OxHb), which is formed by binding oxygen from the air chamber with hemoglobin (Hb) in the blood. So, if a sample tube has little whole blood volume (or it has a large air chamber), more oxygen can bind to the Hb of the erythrocytes, so more OxHb are formed and more ethanol is oxidized. However, the table shows that this loss of ethanol is not linear with respect to storage time, so it can be deduced that there are more factors that contribute to this loss. These losses could be due to volatilization of ethanol, presence of microorganisms that consume alcohol...3. Loss of ethanol is independent of the initial concentration of alcohol. **Conclusion:** It is clear, that in all samples there is a loss of ethanol. Possible solutions to avoid these losses could be filling the samples tubes up, which it would avoid the air chamber; using urine or serum samples, avoiding the presence of erythrocytes and also the presence of hemoglobin; and, finally, freezing the samples at -20 ° C, with which it prevents the oxidation process.

B-396

High throughput SPE and LC-MS/MS Methods for drugs of abuse in Urine

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Objective

Drugs of abuse are commonly tested in clinical labs by dilute and shoot (D&S) approach, associated with high matrix interference at low concentration and long-term deterioration of the LC-MS system. We evaluated the solid phase extraction with smaller bed weight plate and reduced processing time.

Procedures

Human urine samples were fortified with standards (over 40 drugs of abuse) at different concentrations. For the enzyme hydrolysis recovery test, human urine samples were fortified with codeine-6-β-D-glucuronide, morphine-3-β-D-glucuronide and 6-MAM at ULOQ level (2000 ng/ml of free drug) or with THCA-glucuronide at HQC level (200 ng/ml of free drug). Optimized SPE method for barbiturates and THCA: 0.1 ml of fortified urine was mixed with 0.05 ml of internal standard solution (IS) in 90% methanol, and 0.1 ml of pre-made mixture of 200 mM ammonium acetate pH 6.8 buffer and beta-glucuronidase (BG) solution in 5/2 v/v ratio and incubated at 55ºC for 30 min. A Panthera Deluxe SPE plate (30 mg/well) was pre-conditioned with 1 ml of methanol followed by 1 ml of water. Hydrolyzed urine solution was loaded onto the extraction plate, followed with washing with 1 ml each of water and 20% methanol. The analytes were eluted with 1.5 ml of methanol. Solvent was evaporated under nitrogen at 45ºC and the analytes were reconstituted with 1.5 ml of 30% methanol and analyzed by Gazelle C18 UHPLC column. SPE method for main drug panel: A mixture of 0.4 ml of master mix (ammonium acetate buffer, 25 µl of BG100 glucuronidase and IS) and 0.4 ml of fortified urine was incubated at 68ºC for 30 minutes. Hydrolyzed urine solution was loaded onto the Panthera Deluxe SPE plate (20mg/well) and then washed with 1 ml each of water and 5% methanol. The analytes were eluted with 0.4 ml of 70% acetonitrile, and diluted with 0.6 ml of water, then injected into the Gazelle Biphenyl UHPLC column. An ExionLC-API4500 QQQ MS was operated in negative ion mode for barbiturates and THCA and in positive ion mode for the main drug panel.

Results

Panthera Deluxe SPE gives better reproducibility and recovery than C18 and other types of polymer SPE phases. The extraction recoveries were in the range of 74.2-116.9% for all drugs. Under current hydrolysis conditions, recoveries of all glucuronides were above 89%. Compared to D&S, the Panthera Deluxe procedure gave overall better results demonstrating greater AMR and lower LLOQ. Results for xenobiotic interference/effect testing and matrix induced ion suppression/enhancement were comparable between procedures, while Panthera SPE showed superior results in terms of matrix interference/effect tested at cut-off concentration.

Conclusion

Fast and reliable SPE methods were developed for analysis of over 40 drugs of abuse.. These improved methods demonstrate reduced matrix effects and expand the lower end of the AMR by almost an order of magnitude. The method for main drug panel eliminated evaporation procedure; the processing time per plate is less than 10 minutes maintaining the total cost of sample preparation at a very competitive level.

B-397

Performance Evaluation of the Atellica CH Acet, Dgn, Li, and Sal Assays

J. T. Snyder, K. Estock, C. Tyler, J. Koellhoffer, J. Cheek. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH Acetaminophen (Acet), Digoxin (Dgn), Lithium (Li), and Salicylate (Sal) Assays on the Atellica CH Analyzer. Measurement of these assays is useful for assessing overdose and monitoring therapeutic use. The Acet assay uses the enzyme acyl-amidohydrolase and manganese ions to cleave the amide bond of acetaminophen and form a colored compound. The absorbance intensity of the compound is directly proportional to the amount of acetaminophen in the sample. The Dgn assay uses a digoxin-latex complex, with which digoxin in the sample competes for binding sites of an anti-digoxin antibody. The rate of agglutination is inversely proportional the amount of digoxin in the sample. The Li assay is based on the complexation of lithium ions with a lithium-specific chromoionophore, which produces a direct, colorimetric reaction. The Sal assay uses the enzyme salicylate-hydroxylase and NADH to prompt a decrease in absorbance that is proportional to the amount of salicylate in the sample. **Method:** Performance testing included precision and accuracy. Assay precision was evaluated using the 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 patient-sample results compared to results from the ADVIA® 1800 Clinical Chemistry System. **Results:**

Assay	Within-Lab Precision Range (%CV)	Sample Type	
Acet	1.5-3.7	Serum/Plasma	
Dgn	2.0-8.2	Serum/Plasma	
Li	1.0-2.1	Serum/Plasma	
Sal	0.5-2.9	Serum/Plasma	
Assay	**Regression Equation**	**r**	**Comparison Assay**
Acet	y = 0.97x - 0.1 mg/dL	0.998	ADVIA 1800 Acet
Dgn	y = 0.97x + 0.18 ng/mL	0.994	ADVIA 1800 Dig
Li	y = 0.98x + 0.02 mmol/L	0.997	ADVIA 1800 LITH
Sal	y = 1.01x - 0.9 mg/dL	0.997	ADVIA 1800 Sal

Conclusions: The Atellica CH Acet, Dgn, Li, and Sal Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

B-399

Utilisation of Biochip Array Technology for Detection of Fentanyl and Opioid Novel Psychoactive Substances in Urine

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Background: The use of Novel Psychoactive Substances (NPS) is cause of health concern. Manufactured as synthetic alternatives to traditional drugs, NPS often exhibit similar effects but with heightened potency and legally evasive potential. The increasing number of these drugs represents a challenge in clinical test settings trying to maximise the detection of a large number of these compounds in a sample. Biochip array technology allows the multi-analytical screening of NPS and related analytes from a single sample. By employing simultaneous immunoassays, this technology increases the detection capacity, which is important when facing this opioid epidemic. Rapid development of such assays is also necessary to ensure relevance in a market which is constantly changing. The objective of this study was to evaluate a biochip array, which enables the simultaneous detection of fentanyl and opioid novel psychoactive substances from a single urine sample. Analytes to be detected include: furanyl fentanyl, acetyl fentanyl, carfentanil, ocfentanyl, AH-7921, MT-45, U-47700, W-19, etizolam, clonazepam, mitragynine, buprenorpnine and naloxone.
Methods: Competitive chemiluminescent immunoassays defining discrete test regions on a biochip and applicable to the Evidence series analysers, were employed. The measuring range for each assay were: furanyl fentanyl 0-21.8ng/mL, acetyl fentanyl 0-21.8ng/mL, carfentanil 0-2.5ng/mL, sufentanil 0-5ng/mL, ocfentanyl 0-21.8ng/mL, AH-7921 5ng/mL, MT-45 15ng/mL, U-47700 80ng/mL, W-19 0-40ng/mL, etizolam 0-10ng/mL, clonazepam 0-15ng/mL, naloxone 0-20ng/mL, norbuprenorphine 0-5ng/mL and mitragynine 0-10ng/mL. Recovery at concentrations -50% of cut-off, cut-off and +50% were assessed in human urine to determine inter assay precision (n=18) and validate cut-offs. Intra assay precision was assessed by running precision material replicates. Assay sensitivity was assessed by running negative urine samples (n=20). Results are semi quantitiative
Results: Cut-offs validated for this array were: furanyl fentanyl (1ng/mL), acetyl fentanyl (1ng/mL), carfentanil (0.25ng/mL), sufentanil (1ng/mL), ocfentanyl (2ng/mL), AH-7921 (1ng/mL), ocfentanyl (2ng/mL), AH-7921 (1ng/mL), MT-45 (2ng/mL), U-47700 (10ng/mL), W-19 (2ng/mL), etizolam (2ng/mL), clonazepam (2ng/mL), mitragynine (1ng/mL), naloxone (1ng/mL) and buprenorphine (0.5ng/mL). Recovery (%) was achieved at the tested concentrations within a 70-130% range excepting mitragynine. Mitragynine showed slight over recovery below the cut-off however all replicates spiked at -50% below the cut off reported negative correctly. Inter and intra assay precision were less than 20% for each assay and the limit of detection was less than 50% of the cut-off concentration in each case.
Conclusion: This biochip array, by simultaneously screening fentanyl and opioid NPS from a single urine sample is relevant for the current NPS market, doubling as both a screening method and indication of treatment. It is an anticipated answer for many laboratories facing the crisis of unknown drug combinations and concentration. The array allows rapid and accurate detection of multiple low concentration NPS in a single sample.

B-400

An Evaluation of Analytical Performance of Therapeutic Drugs on the Roche Cobas 8000 Modular Analyser Series

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Background
Therapeutic drug monitoring is a laboratory practice which measures and monitors the concentration of drugs with narrow therapeutic ranges at timed intervals. Determination of serum or plasma drug levels is important to individualise drug dosage to achieve optimum therapeutic efficacy and minimize toxicity. In July 2016, our institution's Department of Laboratory Medicine upgraded the automated chemistry platform to the Roche Cobas 8000 system (Roche Diagnostics, Switzerland) interfaced with the fully automated pre-analytic system Roche Cobas 8100 and the post-analytic system Roche Cobas p501. To minimise laboratory process wastage and overcome manpower shortage, the department intends to move the TDM measurements from our current Roche Cobas Integra 400+ analyser to the c8000 system. We evaluated the analytical performance of nine therapeutic drugs assays, including acetaminophen (ACM) and salicylate (SAL) on the c702 module, and amikacin (AMIK), carbamazepine (CBZ), digoxin (DIG), gentamicin (GENT), phenobarbital (PHNB), theophylline (THEO) and valproic acid (VALP) on the c502 module of the c8000.
Materials and Methods
A correlation study was conducted using human serum or plasma samples (n=40) with concentrations distributed over the analytical measurement range of each therapeutic drug assay. The specimens were analysed on both the I400 and c8000 analysers and results compared. The repeatability and intra-laboratory imprecision of each TDM assay were evaluated using 3 levels of quality control (QC) materials. Each QC level were analysed three times a day (once in the morning, afternoon and night) for five consecutive days. The linearity of the assays was validated using six levels of serum or plasma samples prepared by mixing sera of known high and low concentrations.
Results
The coefficient of variation (CV%) values for repeatability and intra-laboratory imprecision ranges for ACM:1.2-1.9 and 2.61-3.8 (12.07-102.08 µg/mL), SAL:1.5-2.2 and 1.83-2.2 (40.21-466.23 µg/mL), AMIK:1.2-3.6 and 1.3-3.8 (4.79-27.99 µg/mL), CBZ:0.8-2.4 and 1.0-2.5 (3.23-15.9 µg/mL), DIG:1.1-4.8 and 1.5-5.6 (1.02-3.49 ng/mL), GENT:1.9-3.9 and 1.97-4.5 (1.73-6.84 µg/mL), PHNB:2.3-5.5 and 2.5-6.1 (9.67-48.01 µg/mL), THEO:1.2-1.6 and 1.7-2.3 (5.69-30.38 µg/mL), VALP:1.6-2.8 and 2.97-5.6 (34.72-112.34 µg/mL) were within the acceptable range established by manufacturer, except for those of QC level 1 for phenobarbital with CV% of 5.5 and 6.1 (9.67 µg/mL) compared to that of the manufacturer's established CV value of ≤5%. Linear regression between I400 and c8000 for the nine assays are as follows: ACM: y=0.99x-0.72, SAL: y=1.03x+1.15, AMIK: 1.04x-0.23, CBZ: y=0.93x+0.67, DIG: y=0.96x-0.04, GENT: y=0.83x-0.05, PHNB: y=1.03x-0.39, THEO: y=0.99x+0.1 and VALP: y=0.97x+0.17. In the correlation study, I400 and c8000 demonstrated good concurrence with r^2 values of ≥0.98 for all 9 therapeutic drug assays. The percentage of recovery for all the therapeutic drugs was within the allowable linearity limits (≤ ±10%) established by the manufacturer.
Conclusion
Our evaluation data showed that the analytical performance of therapeutic drugs assays on c8000 showed good correlation with the I400. Current reference ranges can also be applied for new assays performed on the new instrument. The incorporation of TDM analysis eliminates the likelihood of human error in pre-analytical phase and increases productivity of the laboratory.

B-401

The incidence of vancomycin-induced nephrotoxicityThe incidence of vancomycin-induced nephrotoxicity in Hong Kong Chinese

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Background: The vancomycin-induced nephrotoxicity (VIN) in Hong Kong was lack of systematic population-wide study until now. This study was to explore the incidence of VIN and identify the characteristics of susceptible patients and the most likely risk factors.
Methods: A retrospective study was conducted using the Hong Kong Hospital Authority Clinical Data Analysis and Reporting System (CDARS). All the data of patients with vancomycin prescription and measurement from 2012 to 2016 in Hong Kong were retrieved from CDARS. With the use of Modified RIFLE criteria, patients with acute kidney injury (AKI) were identified. Patients who had no baseline and follow-up concentration of creatinine, vancomycin treatment <3days or trough concentration not at a steady state were excluded. Results were analyzed by using SPSS version 24. Logistic regression was used to identify the predictors for VIN.

Results: Twenty-three patients were identified as VIN from 140 complete cases in Hong Kong from 2012 to 2016. The cumulative incidence of VIN was 16%. From 2012 to 2016, the incidence was 9%, 23%, 26%, 11% and 13% respectively. There were no significant differences between VIN and non-VIN groups in their demographics. No significant association was found between vancomycin levels and the occurrence of nephrotoxicity. In logistic regression analysis, only length of stay had a significant positive association with VIN (odds ratio 1.020, 95% CI 1.004-1.035). **Conclusion:** The incidence of VIN in Hong Kong is low but shows no decline. Long hospital stay is a risk factor for VIN.

B-402

A rapid ultra-performance LC-MS/MS assay for determination of serum unbound fraction of voriconazole in cancer patients

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Background: Voriconazole (VOR), an antifungal agent, is used in the curative treatment of invasive fungal infections and also the prophylactic treatment of opportunistic fungal infections in immunocompromised patients. In blood, VOR is highly protein-bound and mostly with albumin. Free or unbound VOR is the pharmacologically active form. Increased unbound VOR produces high therapeutic efficacy but also enhances toxicity especially in liver or renal failure patients with decreased albumin levels and drug clearance. Monitoring of VOR is thus, highly recommended. In particular, serum unbound VOR should be measured to provide the most accurate monitoring of VOR therapeutic efficacy and toxicity. Setting the appropriate dose for active VOR is challenging due to its variable unbounded forms. We developed and validated an accurate, simple, fast, and cost-effective test with ultrafiltration and UPLC-MS/MS to measure unbounded VOR in human serum for patient testing in clinical laboratories in addition for the measurement of total serum concentrations of VOR. **Methods:** Agilent ultra-performance liquid chromatography (UPLC) system coupled with a SCIEX QTRAP4000 mass spectrometer was performed with a positive ionization mode. Total analytical run time was 3 min. **Results:** All analyses demonstrated linearity (r^2>0.998) from 0.1 to 10 µg/mL for total VOR, while 0.02 to 2.5 µg/mL for unbounded VOR, acceptable accuracy and precision (%CV<15%), suitable stability under relevant storage conditions. Serum samples from sixty cancer patients were collected and both total and unbounded VOR were measured. The levels of total VOR were correlated well with reference laboratory results. The fraction of unbounded VOR was about 2.55% of the total (1.11-4.69%). The levels of serum total and unbounded VOR were highly correlated (r=0.86, p<0.0001). There was a negative correlation between unbounded VOR fractions and levels of plasma albumin (p<0.05). In cancer patients whose albumin levels were low, the fraction of unbounded VOR was high, suggesting that the unbounded VOR should be measured for TDM, even though their total VOR levels were not high. **Conclusion:** In conclusion, a simple and rapid UPLC-MS/MS method for monitoring unbounded VOR is developed and this assay is suitable for routine therapeutic drug monitoring in clinical laboratories.

B-403

Identification of microRNA-mRNA networks involved in cisplatin-induced renal tubular epithelial cells injury

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Background: Cisplatin is a widely used chemotherapeutic drug that often causes acute kidney injury (AKI) in cancer patients. The mechanism of cisplatin-induced renal damage is not completely understood. Moreover, the contribution of miRNAs to the cisplatin nephrotoxicity remains largely unknown. Here we performed an integrative network analysis of miRNA and mRNA expression profiles to shed light into the underlying mechanism of cisplatin-induced renal tubular epithelial cell injury. **Methods** The human renal tubular epithelial cells HK2 were treated with cisplatin, and then FCM and MTT were used to detect cell apoptosis and viability. In addition, qRT-PCR and western blot were used to detect the mRNA and protein expression levels of the apoptosis related genes. Furthermore, miRNAs and mRNA chip were carried out to identify cisplatin-regulated miRNAs and its' target genes using a cisplatin-induced cell model. Quantitative real-time PCR was applied to validate several differentially expressed miRNAs. Lastly, further bioinformatics analysis including GO/pathway and networks analysis were performed to elucidate the possible biological functions of the differentially expressed miRNAs. **Results:** Microarray analysis identified 47 differentially expressed miRNAs, among them 26 were upregulated and 21 were downregulated. Moreover, integrating dysregulated miRNAs target prediction and altered mRNA expression enabled us to identify 1181 putative target genes for further bioinformatics analysis. Gene ontology (GO) analysis revealed that the putative target genes were involved in apoptosis process and regulation of transcription. Pathway analysis indicated that the top upregulated pathways included MAPK and p53 signaling pathway, while the top downregulated pathways were PI3K-Akt and Wnt signaling pathway. Further network analysis showed that MAPK signaling pathway and apoptosis with the highest degree were identified as core pathways, hsa-miR-9-3p and hsa-miR-371b-5p as the most critical miRNAs, and CASK, ASH1L, CDK6 etc. as hub target genes. **Conclusions:** The integrative analysis combining miRNA and mRNA expression profiles, and the related cellular pathways revealed that miRNAs regulated cell apoptosis and stress response pathways played a crucial role in cisplatin-induced renal tubular epithelial cell injury. Overall, our results provide the molecular basis and potential targets for the treatment of cisplatin-induced AKI.

B-404

Performance Evaluation of the Atellica CH Mycophenolic Acid Assay*

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Background: The purpose of the investigation was to evaluate the analytical performance of the Syva® Emit® 2000 Mycophenolic Acid (MPA) Assay on the Atellica® CH Analyzer. Measurement of the immunosuppressant drug MPA is used to monitor levels and prevent toxic levels in transplant recipients. The assay is a homogeneous-enzyme immunoassay technique. The MPA in a patient sample competes with MPA labelled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme for antibody-binding sites. Enzyme activity increases when the enzyme is not bound to an antibody. The active enzyme converts NAD+ to NADH, and the absorbance is measured spectrophotometrically. **Method:** Performance testing included: precision, linearity, and method comparison. Assay precision, linearity, and method comparison were analyzed using MICROSOFT EXCEL 2010. Precision was tested using 20 replicates run on one day using calibrator and QC samples. Linearity was tested using 5replicates each for a total of nine samples across the assay range. Linearity samples were also tested on the Viva-E® Drug Testing System as a method comparison study. **Results:** The within-run precision ranged from 0.83.8% CV on the Atellica CH Analyzer. The linearity study yielded a regression equation of y = 1.00x - 0.08 µg/mL with r = 1.000 when tested on the Atellica CH Analyzer. The method comparison study yielded a regression equation of y = 1.07x - 0.21 µg/mL with r = 1.000. **Conclusions:** The Atellica CH MPA Assay demonstrated acceptable precision and linearity results when tested on the Atellica CH Analyzer. Method comparison results showed acceptable agreement with an on-market comparative analyzer. *Under development. Not available for commercial sale.

B-405

Performance Evaluation of the Atellica CH Emit Drugs-of-abuse Assays

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Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH Emit® Drugs-of-abuse Assays on the Atellica CH Analyzer. These assays include Amphetamines (Amp), Cocaine Metabolite (Coc), Benzodiazepines (Bnz), Barbiturates (Brb), Propoxyphene (Ppx), Methadone (Mdn), Opiates (Op), Phencyclidine (Pcp), Cannabinoids (Thc), Ecstasy (Xtc), and Methadone Metabolite (EDDP) (MetMtb). Measurement of these assays is used in the determination and semiquantification of illicit drug use. All samples determined to be positive on the Atellica CH Analyzer, relative to a given cutoff value, are confirmed quantitatively by the reference method—gas chromatography/mass spectrometry (GC/MS). The Emit Drugs-of-abuse Assays use a homogeneous enzyme immunoassay technique. The drug in the patient sample competes with drug labelled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme for antibody-binding sites. Enzyme activity increases when the enzyme is not bound to an antibody. The active enzyme converts NAD+ to NADH in the presence of glucose-6-phosphate (G6P), and the absorbance is measured spectrophotometrically. **Method:** Multiple cutoff levels were analyzed for several assays. These include Amp 300 ng/mL (Amp300), Amp 500 ng/mL (Amp500), Amp 1000 ng/mL (Amp1000), Coc 150 ng/mL (Coc150), Coc 300 ng/mL (Coc300), Bnz

200 ng/mL (Bnz200), Bnz 300 ng/mL (Bnz300), Brb 200 ng/mL (Brb200), Brb 300 ng/mL (Brb300), Ppx 300 ng/mL (Ppx), Mdn 150 ng/mL (Mdn150), Mdn 300 ng/mL (Mdn300), Op 300 ng/mL (Op300), Op 2000 ng/mL (Op2000), Pcp 25 ng/mL (Pcp), Thc 20 ng/mL (Thc20), Thc 50 ng/mL (Thc50), Thc 100 ng/mL (Thc100), Xtc 300 ng/mL (Xtc300), Xtc 500 ng/mL (Xtc500), and MetMtb 1000 ng/mL (MetMtb). Performance testing included accuracy. Method comparison studies were evaluated using percent concordance between the Atellica CH Analyzer and Viva-E® Drug Testing System, along with GC/MS for discrepant samples (all Pcp samples were tested using GC/MS), for each cutoff level. **Results:** For the Amp300, Amp500, and Amp1000 assays, the percent concordances were 98.6%, 96.6%, and 97.1%, respectively. For the Coc150 and Coc300 assays, the percent concordances were 98.6% and 97.2%, respectively. For the Bnz200 and Bnz300 assays, the percent concordances were 98.7% and 100%, respectively. For the Brb200 and Brb300 assays, the percent concordances were 91.2% and 100%, respectively. For the Ppx assay, the percent concordance was 96.1%. For the Mdn150 and Mdn300 assays, the percent concordances were 100% and 95%, respectively. For the Op300 and Op2000 assays, the percent concordances were 97.3% and 98.7%, respectively. For the Pcp assay, the percent concordance was 96.4%. For the Thc20, Thc50, and Thc100 assays, the percent concordances were 98.5%, 98.3%, and 98.5%, respectively. For the Xtc300 and Xtc500 assays, the percent concordances were 96.5% and 98.2%, respectively. For the MetMtb assay, the percent concordance was 98.3%. **Conclusions:** Method comparison results showed acceptable agreement with an on-market comparative analyzer.

B-406

Performance Evaluation of the Atellica CH Tacrolimus Assay*

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Background: The purpose of the investigation was to evaluate the analytical performance of the Syva® Emit® 2000 Tacrolimus Assay on the Atellica® CH Analyzer. Measurement of the immunosuppressant drug tacrolimus is used to monitor the therapy of liver and kidney transplant patients. The assay is a homogeneous-enzyme immunoassay technique. The tacrolimus in a patient sample competes with tacrolimus containing glucose-6-phosphate dehydrogenase (G6PDH) enzyme for antibody-binding sites. Enzyme activity increases when the enzyme is not bound to an antibody. The active enzyme converts NAD+ to NADH, and the absorbance is measured spectrophotometrically. Prior to testing, whole-blood samples, calibrators, and controls are pretreated with methanol and a pretreatment reagent. This process lyses the cells, isolates the tacrolimus, and precipitates the majority of blood proteins. The samples are then centrifuged, and the resulting supernatant is used for testing. **Method:** Performance testing included precision, linearity, and method comparison. Assay precision, linearity, and method comparison were analyzed using Microsoft Excel 2010. Precision was tested using 20 replicates run on one day using QC samples. Linearity was tested using two replicates each for a total of nine samples across the assay range. Method comparison was tested on the Atellica CH Analyzer and the Viva-ProE™ Drug Testing System using 18 samples with two replicates each. The first replicate was used in analysis. **Results:** The within-run precision ranged from 3.65.5% CV on the Atellica CH Analyzer. The linearity study yielded a regression equation of y = 0.98x - 0.6 µg/mL with r = 0.997 when tested on the Atellica CH Analyzer. The method comparison study yielded a regression equation of y = 0.98x + 0.2 µg/mL with r = 0.990. **Conclusions:** The Atellica CH Tacrolimus Assay demonstrated acceptable precision and linearity results when tested on the Atellica CH Analyzer. Method comparison results showed acceptable agreement versus an on-market comparative analyzer. **Under development and not available for commercial sale*

B-407

An Evaluation of the New ARK Technologies Methotrexate Immunoassay Method onTwo Automated Chemistry Analyzers

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Background: Methotrexate (MTX) therapy has been approved for a number of malignancies and autoimmune diseases. Unfortunately, MTX toxicities can occur at any dose and require timely rescue using eitherLleucovorin and Gucarpidase to prevent irreversible toxicity. However, accurate and frequent monitoring of MTX plasma concentrations can maximize therapeutic efficacy, improve patient safety, and prevent unnecessary adverse outcomes. In anticipation of future unavailability of Abbott TDx Methotrexate II assay, we evaluated the new ARK Technology methotrexate assay run to two different chemistry analyzers: the Beckman DxC 700 AU and Siemens ADVIA 1800. **Objectives:** Determine the precision and accuracy the new MTX immunoassay run on both the Beckman DxC 700 AU and Siemens ADVIA 1800 automated chemistry analyzers using our traditional fluorescent polarization method on the Abbott TDx as a reference method. **Methods:** Analytical precision was determined using known patient plasma samples of low (n = 3), intermediate (n = 5), and high (n =3) MTX concentrations over a period of 5 days. Analytical accuracy was assessed using the ARK Technology methotrexate immunoassay deployed on both Beckman DxC 700 AU and Siemens ADVIA 1800 chemistry analyzers compared to reference method (Abbott TDx Methotrexate II). **Results:** The ARK MTX Immunoassay method on both the Siemens ADVIA 1800 and Beckman DxC 700 AU showed good-to-excellent correlation when compared to our reference TDx MTX method (Siemens Advia 1800; n=86, y = 1.038x + 0.020; R^2 = 0.971; Beckman DxC, n=86, y = 1.081x + 0.0160; R^2 = 0.952). However, for higher MTX values, the Beckman DxC method showed a consistent negative bias. While both methods showed acceptable accuracy at low concentrations, accuracy was unacceptable for samples with MTX levels below the manufacturer's suggested reportable range. Precision with the ARK MTX Immunoassay using the Siemens ADVIA 1800 platform showed an average coefficient of variance of 3.93% (ranging from 1.10% to 8.89%). **Conclusion:** While the ARK Technology methotrexate assay using either the Beckman DxC 700 AU or Siemens ADVIA 1800 showed good-to-excellent correlation with our reference TDx method, higher MTX levels showed a consistent negative bias on the Beckman DxC. Based on the comparable reportable range and the good-to-excellent precision and accuracy compared to our reference method, we conclude that ARK MTX Immunoassay is an acceptable alternative method on our Siemens Advia 1800 to our previously validated MTX assay on the Abbott TDx.

B-408

Hydrolysis Efficiency Study of Selected Beta-glucuronidase Enzymes for Opioid Measurement in Urine

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Background: β-glucuronidase enzymes are used for hydrolysis of opioid glucuronides in urine for consistent measurements. Originally we used β-glucuronidase from *patella vulgate* (limpets), 1-3.2M U/g in acetate buffer with an 18 hour incubation at 60°C. The aim of this study was to investigate whether other commercially available glucuronidase enzymes may offer better hydrolysis efficiency with a much shorter incubation time. **Method:** Six different studies were performed. In each study two levels of prepared glucuronide urine samples (300 ng/mL and 1000 ng/mL) were extracted with one of the enzymes and incubated at 3 time points (30 minutes, 1 hour and 3 hours). The enzymes studied were β-glucuronidase from E.Coli recombinant from overexpressing BL21, >20M U/g in phosphate buffer incubated at 37°C, IMCSzyme, a genetically modified β-glucuronidase , >50K/mL in phosphate buffer incubated at 55°C, β-glucuronidase from *Haliots refuescens* (red abalone) 1–3.2M U/g incubated at 60°C, and β-glucuronidase from *patella vulgate* (limpets),1-3.2M U/g in acetate buffer incubated at 60°C. One extraction had no enzyme using only phosphate buffer incubated at 55°C. The extraction was completed following the incubation and the aliquots were analyzed by an established HPLC-MS/MS method. **Results:** Codeine showed the lowest degree of hydrolysis across all enzymes and time points. The extractions with only buffer showed no sign of hydrolysis. *Haliots refuescens* had the lowest degree of hydrolysis of all enzymes across all time points. E.Coli had the highest degree of hydrolysis at the 3 hour time point. IMCSzymne had the highest degree of hydrolysis at 30 minutes. **Conclusion:** Both E.Coli and IMCSzyme showed improved hydrolysis efficiency over the limpets enzyme. Codeine glucuronide had the lowest hydrolysis efficiency of all the glucuronides tested. IMCSzyme provided the highest hydrolysis efficiency of codeine at the shortest time point (30 minutes) offering the overall best results.

B-409

Performance Evaluation of Representative Clinical Chemistry Assays from the Therapeutic Drug Monitoring Panel on the Alinity c System from Abbott Laboratories

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Background: The Abbott Therapeutic Drug Monitoring Panel, or TDM, determines drug concentration measurements in body fluids as an aid to the management of drug therapy for the cure, alleviation, or prevention of disease. Routine monitoring ensures that therapeutic drug concentrations are in the right therapeutic range for optimal patient care. Abbott TDM assays are carefully designed to measure drug concentrations of analytes that require strict dosage control under STAT or routine testing conditions. The Alinity ci system is part of a unified family of systems that are engineered

for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 1350 tests per hour. The Alinity ci system has an increased reagent load capacity, holding up to 70 Clinical Chemistry reagents, onboard QC and calibrators, clot and bubble detection ability, and smartwash technology to provide consistent and reliable results. **Objective:** To demonstrate the analytical performance of representative assays from the Therapeutic Drug Monitoring Panel of the Alinity c system, which consists of assays that utilize photometric technology for the quantitative determination of analytes in human serum or plasma. **Methods**: Key performance testing including precision, limit of quantitation (LoQ), linearity and method comparison were assessed per Clinical and Laboratory Standards Institute protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met. **Results:** The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the Therapeutic Drug Monitoring Panel are shown in the table below.

Assay	Total %CV	LoQ	Method Comparison to ARCHITECT (Slope/Correlation)	Measuring Interval
Amikacin	≤5.3	2.3 µg/mL	1.02/1.00	2.3 to 50.0 µg/mL
Theophylline	≤3.7	1.3 µg/mL	0.94/1.00	2.0 to 40.0 µg/mL
Tobramycin	≤3.1	0.3 µg/mL	1.01/1.00	0.3 to 10.0 µg/mL
Valproic Acid	≤2.5	5.1 µg/mL	1.00/1.00	12.5 to 150.0 µg/mL
Phenobarbital	≤5.8	1.9 µg/mL	0.98/1.00	2.0 to 80.0 µg/mL

Conclusion: Representative clinical chemistry assays utilizing photometric technology on the Alinity c system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT clinical chemistry assays.

B-410

Biomonitoring of Chromium (Cr) and Cobalt (Co) for Joint Implant Failures by ICP/MS

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Background: Artificial joint implants are intended to restore mobility and relieve pain. The composition of these implants vary, but the metal-on-metal style carries some additional risks. During movement, metal can be released from the implant into the circulatory system. Adverse effects associated with toxicity of these metals include vision and hearing loss, dermatitis, and myocardial failure. Evaluation of patient risk includes clinical history, physical examination, imaging tests, and laboratory analysis. Because both chromium (Cr) and cobalt (Co) are essential elements, it is suggested that baseline levels are obtained prior to surgery. The objective of this work is to present a validated method for the determination of Cr and Co concentrations in blood, serum/plasma, urine, and fluid specimens. **Methods:** Quantitative determinations of Cr and Co concentrations in blood, serum/plasma, urine, and fluid were performed using an octopole collision cell to reduce matrix interferences from polyatomic species. Internal standards (Sc, Ge and Rh) were added to calibration standards, controls, and samples, and then introduced into the instrument. Analyte concentrations were calculated from the measured ion signal and the internal standard isotope signals using aqueous-based calibration curves. The analytical measurement ranges for Cr and Co are 1.0 mcg/L - 1,250 mcg/L and 0.5 mcg/L - 625 mcg/L, respectively, in blood, serum/plasma, and urine; and 1.0 mcg/L - 625 mcg/L in fluid for both elements. For reference purposes, normal Cr and Co concentrations are typically less than 1.0 mcg/L in blood and serum/plasma specimens. In urine, normal Cr and Co concentrations are typically less than 1.0 mcg/mL and 2.0 mcg/L, respectively. **Results:** Retrospective data analysis of samples submitted for both Cr and Co analyses from July 2014 to February 2018 was completed. The data set consisted of 280 blood samples, 640 serum/plasma samples, 6 urine samples, and 258 fluid samples. In blood, positive Cr (n=171) and Co concentrations (n=198) averaged 4.89 mcg/L (median: 4.80 mcg/L; range: 1.0 - 51 mcg/L) for Cr and averaged 4.89 mcg/L (median: 5.00 mcg/L; range: 0.51 - 66 mcg/L) for Co. In serum or plasma, positive Cr (n=359) and Co concentrations (n=475) averaged 4.97 mcg/L (median: 2.00 mcg/L; range: 1.0 - 120 mcg/L) for Cr and averaged 6.87 mcg/L (median: 3.80 mcg/L; range: 0.50 - 160 mcg/L) for Co. In urine, positive Cr and Co concentrations (n=5) averaged 146 mcg/L (median: 35 mcg/L; range: 3.0 - 390 mcg/L) for Cr and averaged 226 mcg/L (median: 89 mcg/L; range: 9.9 - 790 mcg/L) for Co. In fluid, positive Cr (n=119) and Co concentrations (n=140) averaged 15,301 mcg/L (median: 95 mcg/L; range: 2.5-390,000 mcg/L) for Cr and averaged 3,002 mcg/L (median: 145 mcg/L; range: 1-29,000 mcg/L) for Co. **Conclusion:** Potential health risks are associated with metal-on-metal implants as a consequence of elevated metal levels. Patients with evidence of excessive device wear or a localized adverse tissue reaction should be assessed for systemic effects of exposure to metal ions. The presented method can be used to monitor Cr and Co exposure in patients before and after joint implant surgery in several matrices.

B-411

Development and validation of an UPLC-MS/MS analytical method to quantify voriconazole in human plasma

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Background: Voriconazole is essential to the treatment and prophylaxis of invasive fungal infections. Significant pharmacokinetic variability combined with positive exposure-response relationship has increased demand for therapeutic drug monitoring of voriconazole. Here, we develop and validate a fast and simple ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to measure voriconazole in human plasma, which is suitable for the needs of clinic. **Methods:** Samples were purified via liquid-liquid extraction using methyl tert-butyl ether. Cyproheptadine was used as the internal standard (IS). The optimal chromatographic behavior was achieved on a BEH C18 column (1.7 µm, 2.1 mm× 50 mm) using a mixture of 0.3% formic acid in 0.02 mol/L ammonium acetate and acetonitrile (60:40, v/v) as the mobile phase. Mass spectrometric detection was conducted with a triple quadrupole detector equipped with electrospray ionization in the positive-mode using multiple reaction monitoring (MRM). **Results:** The total analytical run time was within 3 min. Our method provided consistent recovery of more than 86.56% for both voriconazole and IS. The calibration curve was prepared, which provided an excellent linearity from 0.5 to 1000 ug/L with a correlation coefficient (r^2) >0.99. The limit of detection (LOD) was determined to be 0.125 µg/L. The intra- and inter-day presicion were 1.81% and 3.57% on average. The overall accuracy were −0.85 ~ 5.14% and 2.20 ~ 4.23% for intra- and inter-day values, respectively. Selectively, dilution integrity and stability were also validated. The method was successfully used to evaluate voriconazole clinical practice in the "real-world" setting. **Conclusions:** A rapid, sensitive and robust UPLC-MS/MS method for quantifying voriconazole levels in human plasma was validated. It was helpful in improving voriconazole related personalized medicine strategies.

B-412

Sample stability and method comparison of a urine benzodiazepine quantitation method by LC-MS/MS

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Background: Benzodiazepines (BZD) are one of the mostly prescribed classes of drug worldwide. They are central nerve system depressant used as sedatives, anticonvulsants, anxiolytics, and muscle relaxants. BZD are frequently been identified in illicit use, mostly for recreational purposes but also been used in certain crimes. BZD is relatively safe in overdose if taken alone; however, there is an increasing number of BZD abuse with opioids among pain management patients and the co-administration will cause excessive suppression to the central nervous system and sometimes leading to death. We have previously reported an LC-MS/MS quantitation method for measuring seven BZD and metabolites (7-aminoclonazepam, α-hydroxyalprazolam, α-hydroxytriazolam, oxazepam, lorazepam, nordiazepam and temazepam). Here, we present study results for sample stability and method comparison. **Methods:** The seven BZD were spiked into negative urine samples to three concentration levels at 100, 1000 and 5000 ng/mL. The spiked samples were then stored at ambient, 4 °C, and -20 °C. Samples were removed and stored at -70 °C when each storage time was reached, and analyzed in triplicate as one batch at the end of the study. The sample storage condition was acceptable if the calculated mean values were within 20 % of the initial values. We compared our LC-MS/MS method to a GC-MS method offered at an independent laboratory using both patient urine samples and spiked urine samples. To further evaluate the accuracy of this method, another comparison was performed using commercial BZD urine toxicology controls (UTAK control) at levels of 100 ng/mL and 400 ng/mL. Percent difference from the UTAK target value and reference value (result from an undisclosed laboratory measurement using LC-MS/MS methodology) were calculated. **Results:** We found 7-aminoclonazepam was less stable with 12 h at both

ambient and 4 °C, 7 days at -20 °C storage. All other six BZD were stable for 24 h at ambient, 14 days at 4 °C, and 3 month at -20 °C. For the method comparison, all negative urine samples tested by our LC-MS/MS method were also been tested negative by the GC-MS method. For the positive urine samples, Deming regression showed correlation coefficient (R) > 0.96 for all analytes. Six of the seven analytes showed positive bias with 7-aminoclonazepam being the highest at 24.5 %, which was most likely due to its instability during shipping and transportation to the other laboratory. Whereas α-hydroxyalprazolam showed a negative bias at -15.4 % and the bias was mainly from the high concentration samples (> 1700 ng/mL by our method). All bias for all the remaining analytes were within 13 %. For the commercial control materials, our LC-MS/MS method showed -15. 8 % to 17.3 % difference from the UTAK target values and -2.7 % to 37.9 % difference from their reference values. **Conclusion:** Additional validation data support the use of this LC-MS/MS method for clinical use.

B-413

Cigarette Smoke And Oxidative Stress-Induced Hypertension

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Background: Cigarette smokers are exposed to significant quantities of reactive oxygen species (ROS) which play a major pathophysiological role in oxidative stress-induced hypertension through the process of lipid peroxidation. The mechanisms underlying oxidative stress-induced hypertension in cigarette smokers are not completely understood. Here, we evaluated the possible effects of cigarette smoke on malondialdehyde (MDA), total antioxidant status (TAS), systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI). **Methods:** A total of 90 male subjects were recruited and grouped into control group (nonsmokers: n=30) and test group (active smokers: n=60). The plasma levels of MDA and TAS were determined; SBP and DBP were measured while BMI was calculated using standard formula. **Results**: A significant increase ($p< 0.01$) was observed when the means of SBP, DBP and MDA in smokers were compared with corresponding control group, while a significant decrease ($p<0.01$) was observed when TAS levels in smokers were compared with corresponding nonsmokers**.** Although there was a subtle increase in the BMI of smokers when compare with nonsmokers, no statistically significant difference was observe. **Conclusion:** The increase in SBP, DBP and MDA concentration coupled with decrease in TAS observed in cigarette smokers indicate that cigarette smokers are at high risk of developing oxidative stress-induced hypertension due to increase lipid peroxidation. **Key words:** Smokers; Oxidative stress; Hypertension.

B-414

Genotyping of selected alleles involved in tramadol metabolism provide evidence for additional factors beyond CYP2D6-inffered phonotype that may contribute to observed metabolite patterns.

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Background: Tramadol is a centrally acting opioid analgesic used to treat moderate to severe nociceptive pain. Structurally, it bears similarities to codeine and morphine, and exhibits similar analgesic potency. The parent prodrug inhibits neurotransmitter reuptake and its bioactivation by CYP2D6 to *O*-desmethyl tramadol (M1) stimulates the μ-opioid receptors, enhancing its analgesic properties. *N*-demethylation by CYP2B6 and CYP3A4/5 renders tramadol inactive by generating *N*-desmethyl tramadol (M2). Interpatient variability in response to tramadol treatment ascribed to polymorphisms in the genes that code for proteins involved in the pharmacokinetics and pharmacodynamics of tramadol. The Clinical Pharmacogenetics Implementation Consortium provides guidelines for CYP2D6-guided therapy for tramadol; however the guideline does not account for additional factors that may contribute to opioid analgesic outcomes including multigene variants and drug-drug interactions. In this study, we have analyzed metabolic patterns for tramadol in serum/plasma and urine for results reported by a national reference laboratory and, for a subset of patients, we determined their genotypes and interrogated the correlation between CYP2D6-inffered metabolizer phenotype and observed metabolic patterns. **Methods**: Concentrations of tramadol, M1 and M2 for 1,321 serum/plasma and 21,686 urine samples were retrospectively analyzed. Testing was performed by LC-MS/MS and 50ng/mL, 100ng/mL and 100ng/mL were used as positive cutoffs for tramadol, M1 and M2, respectively. Positivity for parent drug and M1 or M2 and ratios of M1/M2 were assessed to identify the metabolic patterns. DNA was extracted and selected pharmacogenes involved in tramadol pharmacology (ABCB1, COMT, CYP2B6, CYP2D6, CYP3A4, CYP3A5, and OPRM1) were evaluated using a custom OpenArray genotyping panel assay on the Quant Studio 12K instrument (Thermo-Fisher) for twelve patients taking tramadol. A copy number variation assay was also performed for each patient to identify large deletions or duplications of CYP2D6. For CYP2D6, linear regressions were carried out to compare metabolite concentrations with the predicted phenotype or activity score. Pearson coefficient was determined to evaluate the degree of correlation. **Results:** Presence of parent drug and both metabolites was a common finding for urine samples (90.5%) compared to serum/plasma (13.8%). Parent drug, in the absence of any metabolites was detected for 57.9% of serum/plasma specimens compared to 2.8% in urine. Highest frequency of results was observed at 1.0-1.2 ratio of M2/ M1 with median concentrations of 208 and 178ng/mL (S/P) and 4953 and 5371ng/mL (urine). CYP2D6 genotyping for twelve patients identified nine distinct genotypes with predicted CYP2D6 activity scores ranging from 0.5 to >2. Comparison between predicted phenotypes based on CYP2D6 activity revealed a moderate correlation with M1 (r=0.52), a low correlation with M2 (r=0.33) and no significant correlation with M1/M2 (r=0.14). **Conclusions:** Differences between the CYP2D6-inffered phenotype and tramadol metabolic phenotypes suggest involvement of other factors that contribute to the patient's response to tramadol treatment. This study was limited by lack of clinical information including co-medications and tramadol dosing, but patterns described here showed relatively poor correlations with CYP2D6 genotypes alone. The data highlight the importance of monitoring clinical response, drug-drug interactions and polymorphisms in several genes that may impact tramadol pharmacology.

B-415

Multidisciplinary Approach for Standardized Care and Control of Opioid Administration for Pain Management Patients

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Objective: A multidisciplinary team (Anesthesiology, Behavior Health, Family Practice, Internal Medicine, Pathology, Psychiatry) developed a patient-centered approach to manage chronic noncancer patients in order to minimize the risk of opioid abuse, diversion, and addiction. A clear and concise guide was created for ordering and interpreting pain management drugs to help providers better manage cases and serve patients safely and effectively. **Relevance:** Abuse and addiction to opioid analgesics has become a major patient safety risk in the United States and has worsened over the past few years. According to the Michigan Department of Health and Human Services, in 1999 only 22%of the state's drug overdose deaths were attributed to opioids and heroin abuse. Recently, it was reported as up to 67 percent. **Methodology**: We developed a primary care policy to ensure providers and patients consider the safest and most effective treatment for non-cancer, non-palliative, chronic pain patients. Incorporated into the standard work of medical assistants was a screen for opioid risk of abuse, diversion, and overdose every 12 months. Patients that violate the medication management agreement are flagged with a banner on the medical information system splash page. A chronic pain registry was developed to track patients on the chronic pain syndrome problem list. Patients on the registry trigger health maintenance components, drug screens every 12 months, an automated prescription systems program every 12 months, pain and wellness score every 6 months, and pain treatment contract renewal. A dashboard was created to track providers' use of the pain registry and individual providers are coached for use of the registry. Pathology implemented a new directed chronic pain panel, where a negative opiates screen (and negative cocaine screen) will reflex to a confirmatory opiates (GCMS) order. A drug screen ordering tip sheet was developed to guide screen (qualitative) and confirmation (quantitative) ordering practices. **Validation:** In a survey of primary care providers, 92% requested a streamlined process for prescribing opioids for patients who actually need them. The extent to which providers enrolled their patients into the pain registry was monitored. To examine the effectiveness of the physician education component of our program, we assessed whether narcotics ordering volumes changed. Provider ordering practices were examined before and after focused education regarding proper drug screening and confirmatory strategies. **Results and Conclusions**: Of the 5927 patients being cared for chronic pain during the study period, enrollment by the provider to the registry increased from an average of 22% (median 16%) to 36% (33%). Patient enrollment varied widely by clinic site, from 0% to 73% of their patients. The program resulted in more appropriate test ordering by providers, with 56.9% before program initiation and nearly complete compliance afterward. Correct screening panel ordering increased from 75.7 to 237.9 per month and opiates confirmation testing increased from 11.4 to 19.8 per month. Patients managed by pain clinics has remained relatively constant, while prescriptions for narcotics decreased 4.3% year over year. A standardized approach has been instituted to better manage chronic noncancer patients on opioid drugs.

B-416

Evaluation of the measurement of serum TPMT concentration as a novel method to monitor thiopurine therapy

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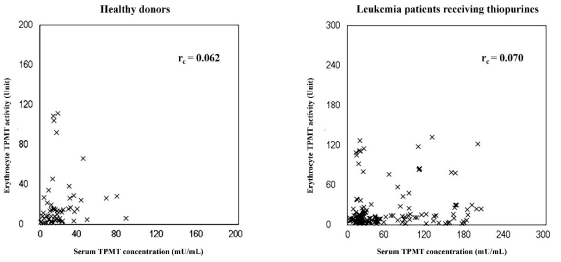
Background:
Thiopurine S-methyltransferase (TPMT) metabolizes thiopurine drugs which are used in various disciplines as immunosupressors and anti-cancer drugs. Decreased activity of the enzyme poses a high risk of severe adverse drug reactions which can be prevented by prescribing decreased thiopurine doses if enzyme status is identified by a prior testing. Currently available methods of TPMT status evaluation are the measurement of the enzyme activity in red blood cells (RBC) and molecular analysis of *TPMT* mutations. *TPMT* genotyping is rather labor-intensive and time-consuming method, while measurement of the enzyme activity is dependent on the RBC condition of the patient which is a matter of concern especially in regions with higher prevalence of hemoglobinopathies. Therefore, considering all the drawbacks of current methods we set up a new study to evaluate the measurement of serum TPMT concentration as a new method.
Methods:
400 healthy blood donors (319 Male/81 Female) and 143 leukemia patients receiving thiopurine therapy (91 Male/52 Female) were included in the study. Measurement of TPMT enzyme activity was chosen as a control method. Serum TPMT concentration (MyBioSource, MBS938845) and enzyme activity (BIOMERICA, REF7019) were both measured by ELISA. Correlation between enzyme activity and serum concentration was analysed via Lin's concordance correlation, and Student's *t*-test was used to analyze between-group differences.
Results:
TPMT serum concentration was higher in female (40.7±10.5) comparing to male (34.5±9.5) donors ($p<0.0001$), and there was a significant increase with the age ($p<0.0001$). Correlation of two methods was $r_c=0.062$ in healthy donors, and $r_c=0.070$ in leukemia patients.
Conclusion:
Measurement of serum TPMT concentration would be a fast and less labour-intensive comparing to *TPMT* genotyping, and it is not dependent on quality and lifespan of erythrocytes and is not influenced by transfusions like the enzyme activity testing. However, the correlation data was not significant enough to support further evaluations of the method.



B-417

BISPHENOL-A EXPOSURE: IMPACT ON HUMAN HELTH

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Introduction: Bisphenol-A, a constituent of plastic and an endocrine disrupting chemical has been implicated to several negative effect on human health.This study was aimed at evaluating some arthropometric measures, oxidative stress indicators and reproductive hormones in males occupationally exposed to Bisphenol-A in a plastic industry in Ibadan, Nigeria. **Methodology:** 80 apparently healthy males aged 18-62 years with normal renal function indices were enrolled into this cross-sectional study. They were forty male employees of a plastic industry (PIW) age matched with forty males who were non-employees of any plastic industry (NPIW, Control). Sexual history, blood pressure (BP), socio-demographic and anthropometric indices were obtained by standard methods. Blood (10mls) was obtained from participants for sex hormones analysis by enzyme linked immunosorbent assay while nitric oxide and superoxide dismutase activities were estimated spectrophotometrically. Bisphenol-A was estimated in spot urine samples using high performance liquid chromatography-tandem mass spectrometry. Data analysed statistically were significant at $p<0.05$. **Result:** Bisphenol- A was detected in both groups but was significantly raised in NPIW compared with PIW ($p<0.003$). NPIW also showed significantly raised diastolic BP and adiposity indices but lower nitric oxide compared with PIW ($p<0.05$). In NPIW, bisphenol-A had a direct relationship with systolic BP and waist circumference but indirect relationship with diastolic BP and waist height ratio ($p<0.05$).No associations were observed between bisphenol-A and physical sexual function indices. However, bisphenol-A had a direct relationship with oestradiol in PIW ($p<0.010$). **Conclusion:** Bisphenol-A was present in both exposed and unexposed groups but was not associated with sexual dysfunction. However, its endocrine disrupting capacity especially in the exposed group is suggested. It's lipophilic nature was shown in the presentations of arthropometric measures and oxidative stress markers in this study.
Keywords: Bisphenol-A, Exposure, Industrialisation

B-418

Ethylene Glycol Elimination Kinetics in an Infant

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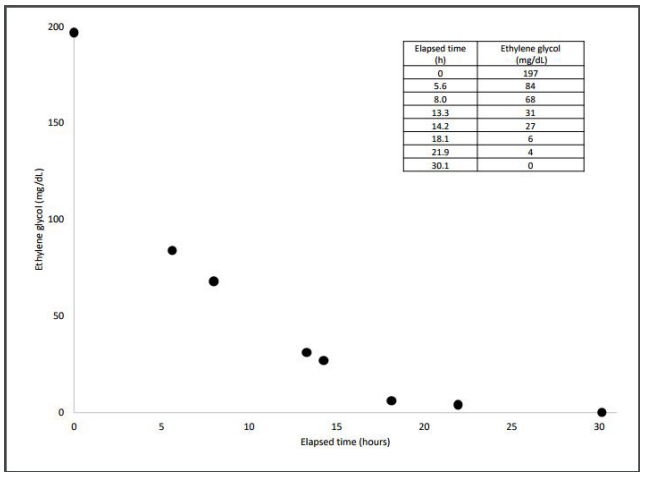
Background: Ethylene glycol (EG) is an ingredient in many industrial products, most commonly found in antifreeze. When consumed, EG is metabolized to toxic metabolites that may result in an elevated anion gap metabolic acidosis (AGMA), renal failure, and potentially death without appropriate therapy. EG toxicity has been previously well described; however, the pharmacokinetics of EG metabolism in infants prior to receiving antidotal therapy has not. The objective of our case study was to determine the pharmacokinetics of EG in an infant. **Methods:** A four-month old infant was brought to the Emergency Department (ED) after one day of altered mental status and vomiting. The patient was noted to be lethargic, tachypneic, tachycardic, and afebrile. Initial laboratory studies were remarkable for a lactate 1.9 mmol/L (17 mg/dL), creatinine 1.93 mg/dL, a $CO2 < 5$mmol/L, and an incalculable anion gap. No infectious source was identified, and the patient was transferred to the pediatric intensive care unit (PICU) at a local pediatric tertiary care hospital. Testing for inborn errors of metabolism resulted on hospital day (HD)2, concerning for EG toxicity. A specimen tested the same day was negative for EG, as determined by gas chromatography with flame ionization detection (Agilent). With a persistent AGMA and worsening AKI, the patient was treated with fomepizole, thiamine, and pyridoxine and continuous renal replacement therapy (CRRT). **Results:** Retrospective analysis of serial specimens from initial presentation showed EG with an elimination half-life of 4.5 hours (Figure). In a single ingestion, an estimated volume of 18 ml of 50% EG containing antifreeze would be required. No other volatiles were detected. The patient subsequently recovered with normal renal function. **Conclusions:** We have uniquely demonstrated the pharmacokinetics of EG in an infant, prior to receiving antidotal therapy to block alcohol dehydrogenase (ADH) activity or hemodialysis, which appears similar to that of adults.



Elapsed time (h)	Ethylene glycol (mg/dL)
0	197
5.6	84
8.0	68
13.3	31
14.2	27
18.1	6
21.9	4
30.1	0

B-419

Therapeutic Drug Monitoring of Lipophilic Immunosuppressive Drugs during Hyperlipidemia

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Background: Therapeutic drug monitoring (TDM) is an integral component for management of transplant patients prescribed immunosuppressive drugs such as cyclosporine, tacrolimus and sirolimus. Due to the lipophilic nature of these drugs, lipemia may interfere with accurate analysis. This investigation was prompted by a cyclosporine order on a grossly lipemic whole blood specimen (>50 mmol triglyceride/L) in a 10 year post-allograft-bone-marrow-transplant patient with polymyositis. Ultracentrifugation is frequently used to overcome lipemia interferences; however this is not appropriate for whole blood specimens and the lipophilic nature of cyclosporine made ultracentrifugation questionable. Furthermore, there is limited data surrounding lipemia interference with our liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. **Objectives:** To investigate whether hyperlipidemia affects LC-MS/MS quantification and the whole blood distribution of cyclosporine, tacrolimus, and sirolimus. **Methods:**
Non-lipemic patient TDM specimens for cyclosporine, tacrolimus, and sirolimus were spiked with a 20% soybean lipid emulsion (Intralipid®) followed by extraction with water, zinc sulfate, and internal standards ([d^{12}]cyclosporine; ascomycin in methanol). Extracts were centrifuged for varying times at 12,000 rpm and by ultracentrifugation. The linearity of hypertriglyceridemic specimens were assessed by dilution with non-lipemic TDM specimens. The *in vitro* distribution of immunosuppressive drug in blood was investigated mixing fresh RBCs with drug-negative lipemic or non-lipemic plasma. Briefly, pooled TDM blood was centrifuged to quantify drug distribution in plasma and washed RBC fractions. Grossly lipemic plasma or non-lipemic plasma were added to washed RBCs (i.e. **lipemic RBCs** and **non-lipemic RBCs**, respectively) and rocked for 24h at 37C. Separately, fresh, non-TDM blood specimens were pooled, and plasma fraction was replaced with equal volumes of either lipemic or non-lipemic plasma. QC material containing cyclosporine, tacrolimus, and sirolimus was added to lipemic (**lipemic+QC**) and non-lipemic (**non-lipemic+QC**) samples prior to 24h incubation. *In vitro* drug distribution was quantified in plasma and RBC fractions after incubation. **Results:** Lipemia did not interfere with LC-MS/MS quantification of cyclosporine, tacrolimus and sirolimus. Ion suppression or chromatographic shifts were not observed; and ultracentrifugation of extracted lipemic specimen in the presence of internal standard did not affect results. Linearity of grossly lipemic blood was acceptable for cyclosporine (R^2=0.99), tacrolimus (R^2=0.98), and sirolimus (R^2=0.99). Whole blood levels of cyclosporine (58 ng/mL), tacrolimus (5.5 ng/mL), and sirolimus (3.8 ng/mL) were largely in RBCs (cyclosporine: 13628 ± 307 ng/10^{12}RBC; tacrolimus: 1477 ± 37 ng/10^{12}RBC; sirolimus: 985 ± 55 ng/10^{12}RBC) versus plasma fraction (cyclosporine: 2.8 ng/mL; tacrolimus: 4.8 ng/mL; sirolimus: 0.01 ng/mL). After 24h incubation, **lipemic RBCs** recovered 36, 66, and 71% of cyclosporine, tacrolimus, sirolimus. In contrast, tacrolimus and sirolimus recovery in **non-lipemic RBCs** was ~105% after incubation, although RBC cyclosporine recovery was 80% in **non-lipemic RBCs**. Lastly, the RBC fraction of **lipemic+QC** contained 64, 80, and 73% of total cyclosporine, tacrolimus, and sirolimus, respectively. Comparatively, cyclosporine, tacrolimus, and sirolimus levels in RBC fraction of **non-lipemic+QC** sample were 70, 94, and 87% of total, respectively. **Conclusion:** Lipemia interferences were minimal in whole blood for cyclosporine, tacrolimus, and sirolimus measurement. However, TDM interpretation of lipophilic drugs should be with caution during hyperlipidemia where drug levels may correlate with lower concentration in RBCs.

B-420

Urine Drug Testing Ranges for Oxycodone, Oxymorphone, and Noroxycodone

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Background: Oxycodone is a semi-synthetic opioid synthesized from naturally occurring thebaine. This strong opioid is commonly used as an alternative to morphine. However, concern about oxycodone is increasing given that oxycodone has similar abuse potential to morphine. Therefore, Urine drug testing (UDT) is often used to help establish whether the patient is indeed adherent to the prescribed drug or is diverting the drug to other purposes. As such, it is important for physicians to recognize drug levels that are outside expected ranges. **Methods:** We present the results of testing a large number of patient urine samples for oxycodone and its metabolites, oxymorphone, and noroxycodone. The data were collected from quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. A data set of 25,259 oxycodone positive urine specimens (50 ng/mL cutoff) collected over a 6 months period (July 2017- December 2017) is used for this study. We separated the data according to the prescribed daily dosages ranging from 5 mg to above 400 mg. We performed Kruskal-Wallis non-parametric test for multiple comparisons. Furthermore, we generated box-whisker plots for the drug concentrations at different daily dosages. **Results:** Kruskal-Wallis test suggested that there are no differences in urine oxycodone concentrations among different groups with daily dosages above 100 mg. When daily doses smaller than 100 mg, the urine oxycodone concentration increased as the dosage increased. Similar trends were also observed for oxymorphone and noroxycodone. **Conclusion:** Drug monitoring results by LC-MS/MS for patients prescribed with oxycodone were used to generated box-whisker plots, giving an expected range for different dosages. The hope is that the data presented herein can aid physicians in determining whether patients are adherent or need additional counseling.

B-421

ARK METHOTREXATE ASSAY ON SIEMENS ATELLICA CH ANALYzER

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BACKGROUND: Methotrexate (MTX), a classical antifolate, can be safely administered over a wide dose range as maintenance chemotherapy for acute lymphoblastic leukemia and treatment of nononcologic diseases including rheumatoid arthritis or psoriasis. When combined with leucovorin (LV) rescue, high-dose MTX (HDMTX; doses of 1,000-33,000 mg/m²) is usually administered as a prolonged i.v. infusion for a variety of cancers, including acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer. HDMTX can be safely administered to patients with normal renal function by vigorously hydrating and alkalinizing the patient to enhance the solubility of MTX in urine. Serum levels may reach 1000 µmol/L or more. Pharmacokinetically guided LV rescue by monitoring MTX serum levels is required to prevent potentially lethal MTX toxicity. Ability to measure MTX accurately at 0.05 µmol/L enables clinical determination of non-toxic status. **OBJECTIVE:** Evaluate the analytical performance of the ARK™ Methotrexate Assay on the Siemens® Atellica CH Analyzer. Compare performance of the assay on the Atellica analyzer to the predicate analyzer (Roche/Hitachi 917). **METHODS:** The ARK™ Methotrexate Assay is a homogenous enzyme immunoassay for quantifying the quantitative determination of MTX in human serum or plasma on automated clinical chemistry analyzers. The assay was evaluated on the Siemens® Atellica CH Analyzer. Increasing reaction rate correlates to increasing MTX concentration for a six point calibration curve (0 to 1.20 µmol/L). Tri-level (0.07, 0.40, and 0.080 µmol/L) quality controls were run. Performance of the assay was determined by assessing precision, limit of quantitation, linearity, analytical recovery, high sample dilution, on board auto dilution, and method comparison. Method comparison samples above the measurement range were serially diluted to within range. **RESULTS:** Total Precision (%CV) for controls was 8.8% (0.07 µmol/L), 3.5% (0.42 µmol/L), and 3.8% (0.81 µmol/L). Limit of Detection (LOD) and Quantitation (LOQ) were comparable to that on the Roche/Hitachi 917: LOD ≤ was 0.02 µmol/L and LOQ was 0.04 µmol/L (0.003 RMSSD, Mean 0.042 µmol/L). Analytical recovery

was within 10% for nominal values >0.10 µmol/L and ±0.01 µmol/L for values ≤0.10 µmol/L. The ARK Methotrexate Aassay was linear from 0.03 to 1.20 µmol/L. High sample dilution was evaluated by performing serial dilutions up to 1:10,000 and resulted in recoveries between 91.7% to 105.0%. Parameters for 1:10 on board automatic dilution with DI water were evaluated and compared to manual dilution using ARK Methotrexate Dilution Buffer. On board dilution resulted in -7.0% to 2.4-5.6% difference from manual dilution. For method comparison, Passing Bablok regression analysis was used. There were 142 samples within measurement range (samples ranged from 0.04 to 1.16 µmol/L): Atellica = 0.95*Hitachi917 + 0.01 (r^2 = 0.97) and 152 samples total including those above the measurement range (samples ranged from 0.04 to 1030 µmol/L): Atellica = 0.95*Hitachi917 + 0.01 (r^2 = 0.99). **CONCLUSION**: The ARK™ Methotrexate Assay performance on the Siemens® Atellica CH Analyzer is substantially equivalent to the Roche/Hitachi 917 analyzer. The ARK™ Methotrexate Assay system on the Atellica analyzer was shown to be safe and effective for its intended use based on performance studies.

B-422

Multiple Drug Classes and Metabolites: Qualitative Analysis in Serum/Plasma by LC-MS/MS

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INTRODUCTION: In emergency department, patients often present with unknown medical conditions and some may present with signs and symptoms of adverse drug reactions. Emergency department laboratory testing may utilize immunoassay or other point of care devices to identify the unknown drugs [1, 2]. However, the numbers of available drugs on immunoassay platforms and POC devices are limited, which may require additional identification and confirmation by GC-MS or LC-MS/MS [2]. **OBJECTIVE:** The purpose of this study was to develop a qualitative method to identify160 drugs/metabolites in serum/plasma by LC-MS/MS to help support testing for adverse drug reactions. This qualitative multi-analyte drug panel consists of different classes of compounds: antiarrhythmic, anticoagulant, anticonvulsant, antidepressant, antihistamine, anti-inflammatory, antipsychotic, barbiturates, benzodiazepines, cannabinoids, decongestant, hallucinogens, hypoglycemic, muscle relaxants, opioids, sedative-hypnotics, stimulants, and tricyclic antidepressants. **METHODS:** PLD+ columns (Biotage) were placed on a 96-well deep plate for extraction. 750 µl of crash solvent that consisted of acetonitrile and a mix of internal standards were aliquoted into each PLD+ column. 250 µl of control, calibrators, or patient sample was added to the appropriate columns, which is an improvement over the previous method requirement of 6 ml of sample. The supernatant was then pushed through the column at $4.1x10^4$-$8.3x10^4$ pascal and collected. The eluted supernatant was then dried, reconstituted, and analyzed on an ABSciex 5500 LC-MS/MS. The run time for the positive mode was 11.5 minutes, and the negative mode was 5.5 minutes. The new method was compared to the current method which utilized a 30 minute GC-MS run, in addition to testing by LC-MS/MS, immunoassay and GC-FID to accommodate a broad spectrum drug screen. **RESULTS:** The cutoffs determined in this assay are analyte specific and ranged from 1 to 100 ng/ml. The presence of a particular analyte above or below the analyte cutoff determines the "present" or "not detected" results of the test. Samples spiked at 50% (n = 4 over 5 days, n = 20 total) of the cutoff screened negative, while samples spiked at 150% (n = 20) of the cutoff screened positive. The LC-MS/MS method had a 70.9% agreement rate with the old extraction and GC-MS method. Further, the new method was able to detect 42.1% more analytes than the old GC-MS method. **CONCLUSIONS:** The new method incorporated an extraction procedure that included a protein crash followed by phospholipid removal. The new LC-MS/MS method enabled the detection of 160 compounds on a single platform, using both positive and negative ionization modes, and reduced the analytical run-time for analysis.

B-423

Therapeutic teicoplanin monitoring reduce the duration of hospitalized days of patients with methicillin-resistant Staphylococcus aureus infections

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Background: Teicoplanin, a glycopeptides antibiotic, has been reported to be comparable to vancomycin in efficacy, but has fewer adverse effects than vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Therapeutic drug monitoring (TDM) of teicoplanin and appropriate loading doses of teicoplanin are required because of potential treatment failure due to suboptimal concentrations of circulating teicoplanin. However, monitoring of teicoplanin concentration in patients is currently not performed routinely in clinical practice in Taiwan. In this study, we conducted a TDM of teicoplanin from patients with MRSA infections. The lengths of patient stay in hospital (LOS) of patients were collected and analyzed. The importance of TDM of teicoplanin in LOS determination was determined. **Methods:** Between July 2016 and July 2017, we prospectively collected 30 blood samples from 12 patients with MRSA infections after five days prescription with three teicoplanin loading doses (12 mg/kg/12 h) followed by maintenance doses of 6 mg/kg/24 h. The teicoplanin concentrations in blood samples were measured by using an immunoassay kit (QMS teicoplanin, Thermo Fisher Scientific). Three other important renal biomarkers, including blood urea nitrogen, creatinine, and cystatin C were also determined in this study. Medical charts of ten patients with multiple teicoplanin results were reviewed and LOS were collected and analyzed. The importance of TDM of teicoplanin in LOS determination from MRSA-infected patients was analyzed by using a multivariate linear regression model. The difference between predictive LOS and true LOS was determined in patients with and without TDM of teicoplanin. **Results:** Among the 12 patients, the teicoplanin concentration ranged from 1.7 to 41.4 mg/L and the average was 19.4 mg/L. Optimal therapeutic concentration (10-15 mg/L) was determined in nine patients and three patients were suboptimal (less than 10 mg/L) during the study period. The LOS of the 10 patients ranged from 9 to 32 days and the average was 21 days. To analyze the importance of TDM of teicoplanin in LOS determination, a multivariate linear regression model was used. The difference between predictive and true LOS was lower than three days if TDM of teicoplanin was applied, and more than five days differences were determined if TDM of teicoplanin was absent. Moreover, the LOS was extended when the teicoplanin concentration was suboptimal. **Conclusion:** Therapeutic teicoplanin monitoring plays an important role in MRSA patient treatment. Maintenance of optimal dosing in the patient during teicoplanin therapy can reduce the duration of hospitalized days.

B-424

Comparing different alcohol markers in routine laboratory testing

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Background: Phosphatidylethanol (PETH), detected in whole blood samples, is like ethylglucuronid (ETG) a metabolite of ethanol and a direct biomarker for alcohol consumption. The verifiability of PETH is longer than the ETG one, but the sensitivity is the same. ETG is detectable in urine as well as in serum. Gamma-glutamyltransferase (γ-GT) and Carbohydrate deficient transferrin (CDT) are indirect biomarker and useful to detect chronical alcohol abuse. A combination of the CDT and γ-GT is the Antilla-Index (AI=0.8 ln(γ-GT)+1.3 ln(%CDT)) with a higher sensitivity and specify as the analytes alone. Aim of the study was to detect and compare results for alcohol consumption with direct and indirect biomarkers in whole blood samples of patients with laboratory request for CDT and γ-GT. **Method**: 111 whole blood samples were analyzed for PETH, ETG, CDT and γ-GT. For positive results the laboratory intern cut offs were used, PETH > 20 ng/ml, ETG >3.2 ng/ml, CDT >2.0%, γ-GT >55 U/L (man) and >38 U/L woman and AI >4.11 (man) and 3.81 (woman). **Results:** Over 50% of the samples have positive results for PETH, but only 30% for ETG, 20% for AI and 10% for CDT. All samples that where positive for ETG, AI and CDT were also positive for PETH. **Conclusion:** The study has shown that determining PETH concentrations in whole blood samples helps to identify alcohol consumption more often than other direct and indirect biomarkers. The problem of all alcohol biomarker is, that it is not possible to find out when the alcohol consumption happened and how high the amount of alcohol consumed was. However a combination of all biomarkers could be a possible way to bring us closer to an answer respectively to frequency and addiction of drinking alcohol.

B-425

Analytical Performance of MyCare Psychiatry Assays for the Detection of Antipsychotic Medications: Risperidone, Clozapine, Arpiprazole, Olanzapine, Quetiapine and Paliperidone

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Background: Adherence to antipsychotic medication is critical to treatment outcomes for patients with mental illness. Measurement of drug blood levels can provide clinicians with objective evidence they need to avoid treatment failures. LC/MS-MS is currently used, however, the time to reported result is days. Optimally clinicians

want to have a reported result sooner. Saladax Biomedical developed rapid immunoassays for the most common antipsychotic drugs to run on random access clinical chemistry analyzers. All measuring ranges are based on recent literature and on established AGNP consensus and Maudsley prescribing guidelines. The objective of this study was to validate assays for the detection of antipsychotic medications in serum. **Methods:** Automated homogeneous immunoassays were developed for risperidone, clozapine, aripiprazole, olanzapine, quetiapine and paliperidone. All assays utilize the same multianalyte calibrators and controls. Analytical performance of each assay was evaluated according to CLSI guidelines using three reagent and three calibrator lots, on two Beckman Coulter® AU480 analyzers. Repeatability and within-laboratory precision were evaluated over 20 days with controls, spiked pooled serum samples, and pooled patient serums. Recovery and Limit of Quantitation were assessed with spiked serum from individual donors. Over 140 prescription and OTC drugs were tested for cross-reactivity. Comparison to a validated LC-MS/MS method was performed with over 400 patient samples. **Results:** The LoQ, linear range, precision, and the Deming regression statistics from the method comparison for each assay are shown in the table below. Co-administered and common prescription medicines and supplements caused less than a 10% bias in the assays' results. All method comparisons between analyzers and lots resulted in slopes of 1 ± 8%.

		Median Imprecision of Serum Pools (CV)		Method Comparison				
Analyte	Measuring Range	Repeat-ability	Within-Labor-atory	R	N	Slope	Inter-cept	Range (ng/mL)
Risperidone	16 - 120 ng/mL	2.4%	4.0%	0.96	146	0.98	1.2	16 - 118
Clozapine	75 - 1423 ng/mL	2.2%	4.8%	0.92	120	0.97	-2.7	80 - 1317
Aripiprazole	25 - 1,000 ng/mL	7.0%	10%	0.99	50	0.94	9.3	29 - 802
Olanzapine	5 - 125 ng/mL	1.5%	2.6%	0.98	82	1.00	2.7	4 - 111
Quetiapine	15 - 700 ng/mL	1.3%	3.2%	0.91	86	1.04	-4.9	15 - 688
Paliperidone	16 - 120 ng/mL	2.8%	4.1%	0.95	119	0.99	2.8	16-120

Conclusion: The MyCare Psychiatry Assays demonstrated robust performance, allowing for rapid, precise, sensitive, specific, and automated measurement of antipsychotic drugs in human serum in clinical chemistry laboratories.

B-426

Can opiate addiction treatment with naltrexone be monitored using saliva?

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Objective. Naltrexone is a potent and safe opiate receptor antagonist used in treatment of opiate addiction. However, patients must be monitored to ensure that therapeutic levels, >2 ng/ml, are maintained. Blood sampling is the preferred method but recovery of a blood sample is challenging in this patient population. We examined the feasibility of using saliva for the purpose of monitoring naltrexone levels. Methods. Healthy subjects (n=7) providing informed written consent were administered a single dose of naltrexone as oral tablet at dose of 25 mg (n=3) or 50 mg (n=4). Blood (serum) and saliva samples were obtained prior to dosing and 1, 3, 5 and 7 hours later. Saliva was obtained using Salivette (Sarstedt). Samples were evaluated by liquid chromatography using tandem mass spectrometer (LC-MS/MS) for detection. Naltrexone assays were evaluated for linearity, matrix effect, stability and interferences. and showed linear response from 0.2 to 1,000 ng/ml. Total protein in saliva was also determined using the bicinchoninic acid assay (BCA assay) method and is linear between 0.5 to 100 ng/ml. Results. One subject was immediately removed from study because nursing staff was unable to obtain a pre-dose blood sample. Serum levels of naltrexone declined in 5 of 6 subjects in a log-linear manner from 1 to 7 hours. One subject showed a steadily increasing naltrexone serum concentration due to slow gastric emptying. Serum concentration of naltrexone (NTX_{Blood}) was shown to be linearly related to saliva concentration (NTX_{Saliva}) by the following equation: $NTX_{Blood} = 0.358 + 0.106 * NTX_{Saliva}$ $R^2 = 0.856$. Saliva concentration of many molecules has been shown to depend on relative hydration state of the individual at the time that a saliva was taken. Total protein, a concentration that changes in response to hydration status, ranged from 1.46 to 4.01 mg/ml showing that hydration status varied significantly. NTX_{Saliva} was divided by total protein to normalize for hydration dependent variation. Normalized saliva naltrexone ($NTX_{Saliva, Norm}$) was regressed against NTX_{Blood}, resulting in the following relationship: $NTX_{Blood} = 1.071 + 0.138 * NTX_{Saliva, Norm}$, $R^2 = 0.892$. A clinical decision rule for increasing naltrexone dose based on $NTX_{Saliva, Norm}$ was explored using Receiver Operator Characteristic (ROC). A clinically important event is when $NTX_{Blood} < 2$ng/ml. ROC AUC = 0.992 for $NTX_{Saliva, Norm}$. Values of $NTX_{Saliva, Norm} < 10$ are associated with false positive and false negative decisions <5%. Conclusion. Obtaining blood from patients undergoing treatment for addiction can be problematic because of poor health and absence of usable peripheral veins. In addition, patients in treatment are often very sensitive to painful stimulus and needles often evoke painful conflicting emotions. Results demonstrate that saliva can be used to monitor therapy with naltrexone. More research is required to assess how naltrexone levels in saliva are related to blood levels in patients undergoing treatment with sustained release naltrexone dosage forms.

B-427

Novel serum biomarker candidates for gastric and/or duodenal ulcers in humans

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Background:
Gastric and/or duodenal mucosal ulceration and hemorrhage are frequently observed side effects in clinical settings. These findings are currently only detectable by endoscopy, and non-invasive diagnostic methods, including biomarkers, have not been identified. Therefore, identifying sensitive and non-invasive biomarkers could facilitate gastric ulcer diagnosis. We previously conducted metabolic profiling of gastric ulcerations induced by aspirin, ibuprofen, ethanol, and stress, and metabolomic analysis of the effects of omeprazole and famotidine on aspirin-induced gastric injury in rats. Based on these studies, we hypothesized that serum hydroxyproline could be a potential biomarker for gastric ulceration. However, we were unable to clarify the usefulness of this biomarker in humans. Here, we confirmed the usefulness of hydroxyproline as a diagnostic biomarker and explored for additional biomarkers through metabolic analysis of sera from gastric and/or duodenal ulcer patients.
Methods:
Twenty-nine patients who suffered gastric and/or duodenal ulcers and were treated with proton pump inhibitors participated in this study. Serum samples were collected four times from each patient during hospitalization or at clinic visits. A total of 509 metabolites in serum were identified and semi-quantified by capillary electrophoresis−time of flight−mass spectrometry (CE−TOF-MS).
Results:
Serum concentrations of metabolites in each sample were categorized into three groups based on the number of days after starting treatment (Group 1: upto3 days, Group 2: between 4 and 14 days, Group 3: from 15 or more days). Metabolite levels were compared among the groups using analysis of variance and Dunnett's test. Statistical analysis showed lower levels of hydroxyproline and higher levels of 2-hydroxybutyrate, 3-hydroxybutyrate, and 2-aminobutyrate in Group 1 compared to Group 3. Therefore, hydroxyproline increased while 2-hydroxybutyrate, 3-hydroxybutyrate, and 2-aminobutyrate decreased with treatment.
Conclusion:
We identified hydroxyproline, 2-hydroxybutyrate, 3-hydroxybutyrate, and 2-aminobutyrate in human serum as potential biomarker candidates for the diagnosis of gastric ulceration. Changes in hydroxyproline in human serum were similar to those observed in a gastric ulceration rat model. Although larger scale studies are needed to confirm the usefulness of these biomarkers, our findings suggest that these new noninvasive biomarker candidates may be useful for gastric injury diagnosis in clinical settings.

B-428

Effect of middleware implementation on LC-MS/MS workflow for immunosuppressant testing

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Background: Middleware is a powerful tool to automatically transfer information from analyzers to laboratory information systems. Although this is routinely in use for main chemistry analyzers, middleware for liquid chromatography-tandem mass spectrometry (LC-MS/MS) instruments has is less available. In the past few years, as demand of LC-MS/MS testing has increased, so has interest in interfacing these instruments. Interfacing has many advantagnes including reduced technologist time to enter results, reduced risk of transcription error and improved turnaround time. Here we describe our experience in implementing middleware on our routine immunosuppressants bench and evaluate the benefits gained in time savings and improved workflow. **Methods:** Middleware from Data Innovations was implemented on the LC-MS/MS bench (Sciex API3000) performing daily immunosuppressant testing (tacrolimus, sirolimus and cyclosporine). An LIS report file (.csv format) was generated from LC-MS/MS software (Analyst) and loaded into the middleware equipped with a Sciex instrument specific driver. The middleware then converted the file into a form acceptable by the laboratory information system (Cerner Millennium). Prior to middleware implementation, the workflow consisted of a manual extraction, manual input of patient accession numbers into the MS software, analysis of data, manual typing of data into the LIS and batch verification of results. Following middlware implementation, accession numbers were scanned into the MS software to make the analytical worklist and all patient results were automatically transferred from the MS software to the LIS through the middleware. **Results:** Prior to middleware implementation, the immunosuppressant bench was struggling with completing the workload each day within a 7.75 hour shift, partially due to the 1-1.5 hours spent per day entering results into the LIS. The extra time gained from middleware implementation has significantly helped in managing the bench workload allowing the technologist to focus on extracting samples and running the LC-MS/MS. Implementation has also had several other positive effects on workflow in the laboratory. For example, as results are no longer manually entered, a second technologist does not need to double check manual transcription into the LIS saving around 20 minutes per day. In addition, the amount of paper printed for record keeping has been significantly reduced by an estimated 3000 sheets per month. And finally, turnaround times have improved on average by 30-40 minutes, which allows for better patient care. **Conclusion:** Middleware implementation has had significant positive impact on the LC-MS/MS workflow in our laboratory

B-429

Multiplexing LC-MS/MS: Addition of Lacosamide to an Anti-Epileptic Panel

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Background Lacosamide is an anti-epileptic used for the treatment of partial seizures. It enhances the slow inactivation of sodium channels, resulting in stabilization of neuronal membranes and inhibition of repetitive neuronal firing. Both LC-MS/MS and immunoassay-based methods are available for measurement of lacosamide. A major technical advantage of LC-MS/MS is the ability to multiplex reactions. In our laboratory, the current anti-epileptic panel consists of 6 compounds: lamotrigine, levetiracetam, topiramate, zonisamide, 10-hydroxycarbamazepine, and rufinamide. In this study, we successfully added lacosamide to the existing LC-MS/MS panel and assessed the performance characteristics of the assay. **Methods** Assay precision was assessed by replicate measurement (n = 12) of lacosamide in patient plasma pools across the concentration range, 4.0 to 20.0 mg/L. The lower limit of quantification (LOQ) was determined by means of the functional sensitivity, assessed by replicate analysis (n = 5) of commercial product pool across the concentration range, 0.5 to 2.0 mg/L. A direct comparison of methods study between our method (Multiplex LC-MS/MS) and a reference method (Reference LC-MS/MS) was performed by measurement of patient specimens (n = 21) across the concentration range, 2.0 to 20.0 mg/L and assessed by Deming-regression analysis. An interference study was performed by testing commercial multi-level therapeutic drug monitoring (TDM) material. **Results** Assay precision was assessed at 2 lacosamide concentrations (mean: 4.8 mg/L and 19.0 mg/L). The mean coefficient of variation (% CV) was 5.3% and 5.7% at 4.8 mg/L and 19.0 mg/L lacosamide respectively. The functional sensitivity of the assay was determined at 1.0 mg/L. Comparison of methods by Deming-regression produced the following values for slope and intercept: Reference LC-MS/MS = 0.037*Multiplex LC-MS/MS + 0.996. **Conclusion** The multiplexing LC-MS/MS method for lacosamide performed with acceptable precision and was comparable to a reference LC-MS/MS method across the therapeutic concentration range. The LOQ value was also acceptable for routine therapeutic drug monitoring. This study has demonstrated that lacosamide can be easily added to an existing LC-MS/MS panel for anti-epileptic drugs.

B-430

Inter-Laboratory Performance of QMSTM Omecamtiv Mecarbil Immunoassay in a Large Global Clinical Study

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Background: Heart failure affects approximately 23 million people worldwide, including more than 5 million in the United States. Omecamtiv mecarbil (OM), a novel selective cardiac myosin activator, is being studied as a potential treatment for heart failure with reduced ejection fraction. Phase 2 clinical study results indicated that a pharmacokinetic (PK)-based dose titration strategy was useful to identify the optimal dose of OM for heart failure patients, a strategy that has been carried into Phase 3 studies. The QMS Omecamtiv Mecarbil Immunoassay was developed for the rapid (time to first result ~ 10 minutes), quantitative determination of OM concentration. The assay is being implemented in central laboratories in the US, EU and Asia to support the OM clinical development program.**Methods:** The QMS Omecamtiv Mecarbil Immunoassay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay. It is based on competition for anti-OM antibody binding sites between omecamtiv mecarbil in the sample and omecamtiv mecarbil coated onto microparticles. A concentration-dependent classic agglutination inhibition curve can be obtained to determine the OM concentration in the sample. The system has two reagent components, six calibrators that span 0 to 1200 ng/mL, as confirmed by a validated LC-MS/MS reference method, and multi-level controls. **Results:** The lower limit of quantification, precision, accuracy, cross-reactivity, potential for interference by endogenous and exogenous substances, method comparison, carry-over and kit stability were found to meet design specifications. The manufacturer's laboratory (TFS) and three central laboratories in US, EU and Asia demonstrated over a twenty (20)-day period lab-to-lab reproducibility less than 3% CV and less than 10% bias versus the validated LC-MS/MS reference method, among other performance characteristics. The results from this study were used to establish quality control limits in a quality control concept that features Westgard Rules and other measures to ensure precision and accuracy. **Conclusion:** QMS Omecamtiv Mecarbil Immunoassay turn-around-time, precision, accuracy and other performance characteristics support its use. CAUTION: As of July 2017, omecamtiv mecarbil (OM), and the QMS immunoassay for omecamtiv mecarbil (OM), are for investigational use only. Limited by United States law to investigational use.

B-431

Retrospective Untargeted and Targeted Urine Drug Screen Trends in Suspected Drug-facilitated Sexual Assault Cases

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Background & Objectives: Drug facilitated sexual assault (DFSA) is the use of drugs to compromise an individual's ability to consent to sexual activity. Drugs commonly identified as used in DFSA include alcohol, flunitrazepam, ketamine and gammahydroxybutyrate. Use of pharmacological agents for DFSA vary significantly, and depend on local availability, ease of use, and the pharmacokinetic properties of drug. Indeed, many cases of DFSA are not confirmed due to a short detection window of certain drugs, or the victim was consuming the drug in moderation (e.g. alcohol). The objective of this study was to investigate retrospective trends in urine drug screen results in suspected sexual assault cases received by our laboratory. **Methods:** Data was obtained from our laboratory information system (Cerner Millennium) for all cases from 2010-2017 under institutional data policies. The dataset included urine drug screen results performed on all patient specimens by an initial immunoassay screen (i.e. amphetamines, benzodiazapines, barbiturates, cannabinoids, cocaine metabolite, ethanol, methadone, methadone metabolite, opiates, oxycodone) followed by untargeted screening and confirmation by gas chromatography-mass spectrometry (GC-MS). Data was analyzed using a custom C++ program. **Results**: 334 suspected sexual assault cases were identified between 2010 and 2017. The number of cases increased from 7 in 2011 to 61 in 2016. 95% of results were from female patients with a median age of 23. Immunoassay screens were positive for ethanol (31%), cannabinoids (22%), cocaine metabolite (19%), amphetamines (16%), benzodiazapines (11%), opi-

ates (7%), and oxycodone (1%). Methadone, methadone metabolite, and barbiturates were present in <1% of cases. GC-MS confirmed the immunoassay screen results for 98% of amphetamines, 30% of benzodiazapines, 68% of cannabinoids, 100% of cocaine, 25% of ethanol, 100% of oxycodone, and 88% of opiate results by immunoassay. Other specific drugs identified by GC-MS including methamphetamine, diphenhydramine and ketamine in 11, 7, and 2% of cases, respectively. Interestingly, 88% of all opioid positive urine specimens were reported since 2014, and 44% of all diphenhydramine positive specimens occurred in 2016. Our results are consistent with previous reports that ethanol remains a commonly detected drug in suspected sexual assault cases. However, flunitrazipam and gammahydroxybutyrate were not detected in this patient population with untargeted GC-MS. **Conclusion**: Real-time monitoring of urine drug screen positivity among suspected DFSA cases may allow the laboratory to target specific drug classes that emerge within specific patient populations. **:**

B-432

UPLC-MS/MS determination of voriconazole in human plasma and its application

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Background: Voriconazole(VCZ), a triazole antifungal agent, was approved for the treatment of invasive fungal infection with a broad spectrum, including Aspergillus Cryptococcus and Candida species. However, a high incidence of adverse reactions may occur during the treatment, such as liver dysfunction and neurological toxicity. Because of the above findings, it has suggested that the blood concentration of VCZ should be maintained between 1.5 and 5.5μg/mL and the measurement of blood levels could assist with decisions about dose adjustment. So we develop a sensitive and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method to determine VCZ concentration in human plasma. **Methods:** We build a simple UPLC-MS-MS method for quantifying VCZ concentration in human plasma, using Cyproheptadine as an internal standard (IS). VCZ and IS were extracted from plasma samples by liquid-liquid extraction with 1 ml of Methyl Tertiary Butyl Ether. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column (2.1*50mm, 1.7um) using an isocratic mobile phase system composed of acetonitrile and 0.02mol/L NH_4Ac containing 0.1% formic acid (40:60, v/v) at a low rate of 0.30 mL/min. Mass spectrometric analysis was performed using a TQ-S mass spectrometer coupled with an electrospray ionization source in the positiveion mode. The multiple reaction monitoring (MRM) mode was used, and the transitions selected for quantification were m/z 350.4→m/z 127.2 and m/z 288.4→ m/z 96.2for VCZ and IS, respectively. **Results:** Good linearity (R^2= 0.9991) was observed throughout the range of 0.0005-10ug/ml in 0.1 ml plasma. The overall accuracy of this method was 99.2-109.5%, and the lower limit of detection was 0.25ng/ml. The intra- and inter-day variations were lower than 3.84% and 6.72%, respectively. Plasma concentrations of VCZ in 793 patients were determined, the blood concentration level of VCZ were between 0.01 and 55.74ug/ml, there are 44.24% VCZ concentration at1.5-5.5ug/ml, 37.45% VCZ concentration below 1.5ug/ml and 18.31% VCZ concentration above 5.5ug/ml. **Conclusion:** A UPLC-MS/MS method for the determination of VCZ in human plasma was developed and validated, This method was rapid, sensitive, specific, selective, reproducible, and successfully applied in therapeutic drug monitoring of VCZ.

B-433

Performance of the ARK Diagnostics, Inc., ARKTM Methotrexate Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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Introduction: The ARK Methotrexate Assay quantitatively determines the concentration of methotrexate in human serum or plasma on automated clinical chemistry analyzers. Methotrexate is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. Methotrexate monitoring helps ensure appropriate therapeutic levels of less than 0.05 – 0.1 μmol/L, and avoid possible toxic effects of the treatment. **Method:** The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between methotrexate present in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. Antibody binding to G6PDH decreases enzyme activity, while binding of methotrexate from the specimen to the antibody reduces antibody bound to G6PDH, thereby increasing enzyme activity. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. The assay was conducted using 8.5 µL of patient sample and the two ARK Methotrexate Assay reagents. Two-point rate measured at 340nm is converted to concentration using a Logit/Log 4 calibration model. Enzyme activity (rate) is directly related to the concentration of Methotrexate in the patient specimen. **Results:** The performance of the ARK Methotrexate assay was assessed on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System. The stated reportable range is 0.04 – 1.2 µmol/L. Linearity testing using an 11 level admixture series resulted in an observed linear range of 0.029 – 1.122 µmol/L. We evaluated accuracy with 50 serum samples (0.09 – 1.04 µmol/L) on the VITROS 4600 and VITROS 5600 Systems compared to the Beckman Coulter AU680 Clinical Chemistry Analyzer. The results show: VITROS 4600 System = 1.052 * Beckman - 0.01388; (r^2) = 0.98. VITROS 5600 System = 1.032 * Beckman - 0.01754; (r^2) = 0.982. A 5-day precision study was conducted on the VITROS 4600 and VITROS 5600 Systems using control fluids at mean methotrexate concentrations of 0.076, 0.418, and 0.852 µmol/L. These resulted in within–laboratory standard deviation (SD) of 0.009 for the low fluid, and within-laboratory percent coefficient of variation (%CV) of 3.36% and 4.25% respectively for the mid and high fluids for the VITROS 4600 System, and 0.007 (SD) for the low fluid and 3.11% and 4.55% respectively for the mid and high fluids for the VITROS 5600 System. The Limit of Quantitation (LoQ) for the VITROS 4600 and VITROS 5600 Systems is 0.04 µmol/L based on 40 determinations per system with 3 samples at 0.03, 0.04, and 0.05 µmol/L methotrexate. The Limit of Detection (LoD) for the VITROS 4600 and VITROS 5600 Systems is 0.023 µmol/L based on 60 determinations per system with 1 low-level sample. The Limit of Blank (LoB) for the VITROS 4600 and VITROS 5600 Systems is 0.005 µmol/L based on 60 determinations per system with 1 blank sample. **Conclusions:** The Methotrexate Assay run on the VITROS 4600 and VITROS 5600 Systems exhibited excellent correlation with the Beckman Coulter AU680 Clinical Chemistry Analyzer, optimal precision and low end sensitivity. RD0082

B-434

A Liquid Chromatography Tandem Mass Spectrometry method for the Simultaneous Screening and Quantification of 10 Analgesics and Narcotics from Micro Plasma Collection Card

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Abstract:
Background: Addiction and abuse of analgesics and narcotics are epidemic worldwide. It is essential to quickly identify and accurately determine those drugs when drug poisoning is suspected. Here we present an application of micro plasma collection card for simultaneous screening and quantification of 10 typical drugs of analgesics and narcotics in plasma by liquid chromatography tandem mass spectrometry method. These drugs include Meperidine, Fentanyl, Morphine, Oxycodone, Tramadol, Acetaminophen, Heroin, Ketamine, Nimetazepam and Methamphetamine. **Methods:** One drop of blood (10-20 microliter) was collected by a micro plasma collection card, and then Dried Plasma Spot (DPS) was extracted before the sample was analyzed by liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF) and liquid chromatography coupled to quadrupole mass spectrometry (LC-MS/MS). The drugs were identified based on retention time and exact mass acquired from molecular ions and fragment ions. After a positive identification by LC-QTOF, the sample was once again quantified by a LC-MS/MS method. Plasma volume factor of the 10 drugs was aquired by calculating the ratio of drug concentrations between DPS and wet plasma from a same blood sample. Hematocrit were evaluated the impact on plasma volume factor. **Results:** All the drugs were well extracted from DPS with recoveries higher than 70%. For LC-QTOF screening method, the limit of detection was 10-50 ng/mL. For the LC-MS/MS quantification method, the accuracy was between 88-113% and precision was less than 10% with linearity curve ranged from 10-1000 ng/mL. Plasma volume factor of each drug was a constant value (from 0.0301 to 0.0597) when hematocrit was between 30-50% or 30-60%. The concentration conversion formula was: Wet plasma (ng·mL-1) =DPS (ng·mL-1) / Volume factor. **Conclusions:** DPS card was a useful tool for convenient and stable biological matrix aimed for screening and quantifying the 10 analgesics and narcotics in human plasma.

Compounds	LOD (ng/mL)	Standard Curve (ng/mL)	Accuracy (%)	Precision (%)	Recovery (%)	Volume Factor	Hematocrit (%)
Meperidine	20	10-1000	88.60-111.3	2.46	81.43	0.0465	30~60
Fentanyl	10	10-1000	90.62-108.7	7.86	84.68	0.0301	30~60
Morphine	50	10-1000	95.20-111.3	6.34	86.39	0.0482	30~60
Oxycodone	10	10-1000	95.40-105.8	4.72	85.91	0.0589	30~60
Tramadol	10	10-1000	97.38-112.3	5.42	81.57	0.0365	30~60
Acetaminophen	50	10-1000	93.00-103.2	9.66	78.84	0.0343	30~50
Heroin	20	10-1000	94.52-105.0	3.48	71.59	0.0401	30~50
Ketamine	15	10-1000	100.2-105.6	5.88	74.29	0.0476	30~50
Nimetazepam	20	10-1000	95.39-104.6	6.09	78.63	0.0329	30~60
Methamphetamine	50	10-1000	97.42-102.5	6.58	80.09	0.0597	30~60

LOD: limit of detection.

B-435

General Unknown Screening of Urine Samples with LC-MS/MS

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Background: Urine drug screening is generally performed in a targeted manner, where analysis is limited to the detection of predefined drugs and metabolites. Untargeted methods for the detection and identification of exogenous molecules, in which analysis is performed in an unbiased manner, have the potential to detect new drugs and unexpected additives. Such a test would be useful for public health purposes, to identify new local trends and aid in investigations of interferents. Here we explore the use of an untargeted LC-MS/MS method combined with database searching to identify exogenous compounds in urine from pain management patients, assessing the identification of drugs previously found by targeted methods and the identification of additional exogenous drugs not previously assessed by available methods. **Methods:** Urine specimens were mixed with acetonitrile, centrifuged to remove particulate matter, then mixed with 0.1% formic acid in water. Liquid chromatography of specimens was performed with a 250 mm pentafluorylphenyl column, using 0.1% formic acid in methanol and 0.1% formic acid in water as the mobile phase with a 30 min gradient. Samples were analyzed in positive mode on a Thermo Q Exactive™ mass spectrometer collecting data-dependent fragmentation spectra, without the use of inclusion or exclusion lists. Data was analyzed using Compound Discoverer™ 2.1 (Thermo) following the forensics unknown workflow, which searches the mzCloud™ database of >7,000 compounds for precursor ion and product ion pattern matches. Commercial drug-free urine was used as a negative control. A mixture of 43 recreational and prescription drugs at 200 ng/mL served as a positive control. De-identified remnant patient samples, obtained from urine toxicology testing, were analyzed. **Results:** Analysis of the positive control with this method detected and accurately identified 38 out of 43 (88%) compounds. An estimate of the limit of detection was obtained by analysis of serial dilutions of this control sample, with most compounds detectable at 20 ng/mL and approximately half detectable at 2 ng/mL. Due to the data-dependent nature the analysis, limits of detection will necessarily vary with the complexity of the sample. Analysis of pain management samples revealed a 72% concordance of the untargeted method with identifications from a validated targeted method, with lack of agreement occurring for analytes present at <100 ng/mL. Additionally, this method was able to identify prescription drugs (e.g. Zyrtec, Nexium, gabapentin) and non-prescription drugs (e.g. psychoactive beetle nut compound arecoline, designer stimulant 4-methoxy-alpha-pyrrolidinobutiophenone) that are not included in the targeted analysis. These results are qualitatively compared to prescription records and patient surveys containing self-reports of drug usage. **Conclusion:** This untargeted LC-MS/MS method, coupled with data analysis with Compound Discoverer™, is able to identify a large variety of compounds, including recreational and therapeutic drugs. We anticipate that this methodology could be used for public health surveillance to aid in the identification of new recreational drug trends, including additives and excipients, and for investigations of potential interferents in targeted assays.

B-436

Performance Evaluation of the New Emit II Plus Oxycodone Assay on the Viva-E System

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Background: Oxycodone is a semisynthetic opioid analgesic prescribed for the relief of moderate to severe pain. Oxycodone structurally resembles codeine and morphine, with similar analgesic properties and potential for addiction and abuse. A new Emit® II Plus Oxycodone Assay for human urine screening has been developed by Siemens Healthineers. The Emit II Plus Oxycodone Assay has cutoffs of 100 and 300 ng/mL. The assay consists of ready-to-use liquid reagents that provide qualitative and semiquantitative results. The data presented in this study was generated on the Viva-E® Drug Testing System. **Methods:** Precision was evaluated at the cutoffs, ±25% controls, and other levels according to CLSI EP5-A2. Analytical recovery was studied by spiking oxycodone into human urine at levels that span the assay range (50-1000 ng/mL). Specimens (100 per cutoff) were analyzed and the results compared to those of LC-MS/MS. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences was assessed by spiking the interferents into human urine in the presence of oxycodone at levels of ±25% of the cutoffs. On-instrument stability was assessed by testing the assay controls over time. **Results:** Evaluation of precision demonstrated qualitative repeatability CVs (rate) for all levels that ranged from 0.18 to 0.36%, and within-lab CVs ranged from 0.41 to 1.32%. Semiquantitative repeatability CVs (ng/mL) ranged from 0.68 to 2.54%, and within-lab CVs ranged from 2.49 to 4.87%. Semiquantitatively, the assay quantified oxycodone-spiked samples between 50 and 400 ng/mL for the 100 cutoff curve and 100-1000 ng/mL for the 300 cutoff curve within ±20% of nominal values. The percent agreement of specimens between the assay run on the Viva-E Drug Testing System and LC-MS/MS was 96% at the 100 ng/mL cutoff and 98% at the 300 ng/mL cutoff. The assay demonstrated 84% detection of oxymorphone in urine relative to oxycodone at the 100 ng/mL cutoff. The assay demonstrated minimal cross-reactivity to structurally related opioids. Potentially interfering substances gave acceptable results relative to the 100 and 300 ng/mL cutoffs. The reagents were stable onboard the Viva-E system for a minimum of 4 weeks. **Conclusion:** The Emit II Plus Oxycodone Assay on the Viva-E Drug Testing System is a suitable screening method for urine specimens at the cutoff levels of 100 ng/mL and 300 ng/mL for both qualitative and semiquantitative analysis of oxycodone.

B-437

Prevalence and Trends in Drug Use: Urine Drug Screening Positivity Rates for Community-based Patients in Ontario, Canada from 2014 to 2017

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Background: Comprehensive multi-year reports detailing the prevalence and annual trends in drug use within a specific patient cohort are often not widely available or current. Urine drug screening positivity rates derived from qualitative liquid chromatography tandem mass spectrometry-based (LC-MS/MS) patient testing may be used to obtain this information. This approach to identifying recent drug use trends in community-based patients in Ontario, Canada has not yet been published. ***Objective:*** Identify multi-year trends in drug use by examining qualitative LC-MS/MS urine drug screening positivity rates. ***Methods:*** All LC-MS/MS urine drug screening results from 2014 (N=136,864), 2015 (N=153,329), 2016 (N=106,687) and 2017 (N=75,774) were retrospectively reviewed. Following enzymatic hydrolysis and protein precipitation, all urine specimens received targeted LC-MS/MS screening which identified the presence of drugs within the following drug classes: anesthetic; anticonvulsant; antidepressant; benzodiazepine; cannabinoid; opioid; stimulant; and illicit. Relevant drug metabolites and related compounds were also included in this test. A total of N=63 different compounds were screened using their respective positive/negative cut-off concentrations. The positivity rates for all analytes were tabulated and partitioned by month of testing. ***Results:*** Over the examined four-year testing period, urine drug screening positivity rates ranged from 76.6% (cotinine) to <0.01% (7-aminoflunitrazepam, desalkylflurazepam, diazepam, flunitrazepam, flurazepam, JWH018, JWH200, MDEA, MDPV, mephedrone and phenazepam). From 2014 to 2017, annual significant ($p \leq 0.05$) increases in urine drug screening positivity rates were observed for: 6-acetylmorphine (0.6% to 1.4%); amphetamine (3.4% to 6.8%); gabapentin (5.4% to 7.6%); and methamphetamine (2.9% to 5.7%). From 2015 to 2017, annual sig-

nificant positivity rate increases were observed for: benzoylecgonine (9.7% to 13.3%); cocaine (2.8% to 4.2%); fentanyl (3.5% to 4.2%); and norcocaine (0.8% to 1.5%). From 2016 to 2017, significant positivity rate changes were observed for: buprenorphine (8.7% to 9.7%); levamisole (8.0 to 5.9%); meperidine (0.08% to 0.04%); methylphenidate (2.0% to 1.7%); naloxone (8.5% to 9.7%); norbuprenorphine (10.0% to 11.1%); norhydrocodone (1.7% to 2.0%); normeperidine (0.1% to 0.07%); and ritalinic acid (3.3% to 2.8%). Relative to the 2017 observed positivity rates, all other analytes included in the LC-MS/MS screening panel did not show significant annual trends or differences within the tested patient population. The 2017 positivity rates for methadone and EDDP were 37.0% and 37.6%. THCA positive rates in 2014, 2015, 2016 and 2017 were 29.6%, 28.9%, 29.5% and 28.6%. ***Conclusions:*** This retrospective review of qualitative LC-MS/MS urine drug screening positivity rates from 2014 to 2017 identified several significant annual changes in licit drug use and provided evidence of an increasing prevalence of illicit drug consumption within the community-based patient cohort. Use of amphetamine, cocaine, fentanyl, heroin and methamphetamine significantly increased but cannabinoid-use was consistent. Use of Suboxone significantly increased in 2017 but evidence of methadone-based opioid antagonist therapy was more common. Laboratories can provide detailed information on drug use trends within a specific patient population by tabulating, interpreting and communicating urine drug screening positivity rates to their clinical communities.

B-438

A novel activity-based concept to screen biological matrices for the presence of (synthetic) opioids

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Background: Highly potent synthetic opioids, which mimic the effects of heroin and morphine, are a growing health threat. Detection of these novel opioids remains challenging as new compounds continue to enter the market. The objective of this study was to set up a novel concept for screening biological matrices for the presence of opiates and (synthetic) opioids, not relying on antibody-based or mass spectrometry-based recognition of the structure of these compounds, but based on their opioid activity. **Methods:** The μ opioid receptor (MOR) belongs to the class of G-protein-coupled receptors (GPCRs). Activation of these receptors results in recruitment of a signaling molecule, β-arrestin 2 (βarr2). We used this principle to set up a bioassay in which we expressed MOR and βarr2, each fused to one part of nanoluciferase, in HEK293T cells. Upon GPCR activation, βarr2 is recruited, which brings both parts of nanoluciferase into close proximity, resulting in its functional complementation, which, after application of a substrate, can easily be monitored via luminescence. In the optimized set-up, applied in 96-well format, HEK293T cells are used that are transiently transfected with plasmids (ratio 4:4:1) encoding respectively: i) MOR, C-terminally fused to the large part of nanoluciferase; ii) βarr2, N-terminally fused to the small part of nanoluciferase; and iii) G-protein coupled receptor kinase 2 (GRK2). Following washing the cells with serumfree medium, 90 µl of medium (Opti-MEM® I) and 25 µl of 20-fold diluted Nano-Glo Live Cell Reagent is added and luminescence is monitored until stabilization. Subsequently, 20 µl of biological extract (reconstituted in medium) is added and luminescence is monitored for 2 hours. Scoring is always done blind-coded. **Results:** Sensitivity and specificity were evaluated using 107 authentic postmortem blood samples with known presence or absence of the synthetic opioids U-47700 or furanylfentanyl, as determined by LC-MS/MS and QTOF analysis. A first finding was that in 8 synthetic opioid positive samples no positive signal was obtained. In these samples, Q-TOF analysis revealed the MOR antagonist naloxone, which can obviously also prevent receptor activation *in vitro*. Hence, evaluation was further based on non-naloxone containing samples. For U-47700 and furanylfentanyl positives, sensitivity was 100% (8/8), respectively 95% (21/22). The missed furanylfentanyl positive sample could not be retested for the presence of naloxone. Of the 59 opioid negative samples, 55 samples were correctly scored negative, yielding a specificity of 93% (55/59). An additional 5 samples (found to contain opioids codeine, (nor) buprenorphine or loperamide) was correctly scored positive. In 5 negatively scored samples, Q-TOF analysis revealed presence of alfentanil (1) or sufentanil (1) (both < 1 ng/ml) or dextromethorphan/levomethorphan (2) or dextrorphan/levorphanol (1) (for the latter, non-detection could be explained by presence of inactive form). **Conclusion:** The MOR reporter assay allows rapid identification of opioid activity in blood samples. Although the co-occurrence of opioid antagonists is currently a (solvable) limitation, the high sensitivity, selectivity and the untargeted nature of the technique may render it a useful first-line screening tool to investigate potential opioid intoxications in clinical and forensic settings, complementing conventional analytical methods which are currently used.

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The Detection and Analytical Confirmation of Synthetic Fentanyl Analogues in Human Urine & Serum using an Ultivo LC/TQ

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Background: During this research study, a sensitive, robust and relatively fast targeted analytical method was developed for the quantitation of 12x synthetic fentanyl opioids, 4-ANPP the synthetic precursor molecule and a similar powerful opioid-like synthetic known as W-18. Simple sample preparation routines were employed to make samples ready for analysis using an Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ) from both human serum and urine matrices. Several separate batches were prepared and analyzed to obtain statistically valid analytical performance results. The lower limits of quantitation, chromatographic precision, calibration linearity, range and accuracy for each synthetic opioid will be presented herein. A comparison of the analytical performance of each analyte for both urine and serum matrices will also be outlined. **Methods:** LC/MS analysis was performed using an Agilent 1290 UHPLC/Ultivo LC/TQ with electrospray ionization (ESI) in positive mode. The chromatographic column used was a Poroshell EC-C18 column (2.1x50mm, 2.7 µm). The UHPLC mobile phases used, A and B respectively, were 0.01% formic acid and 5mM ammonium formate in water and 0.01% formic acid in methanol. Two MRM transitions were monitored for the analytes and a single transition for the deuterated or C^{13} internal standard, during a 7-minute analysis. Human serum samples (250µL) were spiked with calibrators at various concentration levels, cold acetonitrile (500µL) containing the deuterated internal standard was added to affect protein precipitation and centrifuged at 5000rpm. The supernatant was further diluted (1:2) with a 10:90 methanol:water solvent mixture prior to instrument injection. Negative urine was spiked with internal standards and specified calibration levels, centrifuged at 5000rpm (4ºC) for 10 minutes, then 100µL of the supernatant was made up to 1mL in the sample vial by the addition of 900µL de-ionized water. **Results:** Excellent linearity and reproducibility were obtained for human serum extracts typically within an actual concentration range from 10 or 50pg/ml to 500ng/ml (50/250fg to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.997 for three batches. Precision data observed over the three batches resulted with a %RSD variation of <7% across all calibration levels in this research study. Typical results for the diluted urine samples yielded an actual concentration range from 50 or 100pg/ml to 500ng/ml (250/500fg to 2500pg on-column) for each synthetic opioid analyte with a linearity coefficient of >0.996 for three batches. Precision data observed over the three batches resulted with a %RSD variation of <9% across all calibration levels in this research study. In general, the LLOQ sensitivity for each serum-spiked synthetic fentanyl opioid analyte measured in this research exercise was approximately 2x of that obtained from the urine-spiked matrix. **Conclusion:** This research project demonstrates that the performance of theUltivo LC/TQ with the analytical methodology described herein generated excellent linearity, precision and sensitivity across the range of 10 or 50pg/ml through 500ng/ml for each respective synthetic opioid in human serum and sensitivity across the range of 50 or 100pg/ml through 500ng/ml for the respective synthetic opioid in human urine. For Research Use Only. Not for use in diagnostic procedures.

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Activity-based detection of cannabinoid activity in serum and plasma samples

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Background: Synthetic cannabinoids continue to be the largest group of new psychoactive substances monitored by the European Monitoring Center of Drugs and Drug Addiction. The rapid proliferation of novel analogues makes the detection of these new derivatives challenging and has initiated considerable interest in the development of so-called 'untargeted' screening strategies to detect these compounds. Starting from an existing proof-of-concept that worked in urine samples, the objective of this study was to set up an improved activity-based screening assay for the detection of cannabinoid activity in plasma and serum. **Methods:** We previously developed cell-based cannabinoid reporter bioassays for the detection of synthetic cannabinoids and their metabolites, capable of demonstrating cannabinoid activity in authentic urine samples. The principle of these bioassays is activity-based, where activation of the cannabinoid receptors CB1 or CB2 leads to β-arrestin 2 (βarr2) recruitment, which results in functional complementation of a split NanoLuc luciferase, thereby restoring luciferase activity. In the presence of the substrate furimazine, this results in a biolu-

minescent signal, which can be read out with a standard luminometer. Based upon this successful proof-of-concept, we have developed new stable cell lines, in which a truncated rather than a full-length βarr2 molecule was used, with the aim to further improve the assay's performance. This new bioassay was evaluated using extracts of authentic serum (n = 45) and plasma (n = 73) samples. For sample preparation, 500 µl of matrix was subjected to a simple liquid-liquid extraction using hexane:ethyl acetate (99:1 v/v). Following evaporation and reconstitution in 100 µl of Opti-MEM® I/methanol (50/50 v/v), 10 µl of these extracts was analyzed in the bioassays, which were performed in HEK293T cells that stably expressed an optimized combination of either CB1 or CB2, along with a modified βarr2. Scoring was performed blind-coded. **Results:** Truncation of βarr2 significantly (P = 0.0034 and 0.0427 for CB1 and CB2, respectively, unpaired student's t-test) improved the analytical sensitivity over the previously published bioassays, applied on urine samples. For CB1, the best result was obtained when fusing CB1 to the large part of NanoLuc and combining this in the cell system with βarr2, truncated at residue 366 and fused N-terminally to the small part of NanoLuc. For CB2, the best result was obtained when fusing CB2 to the small part of NanoLuc and combining this in the cell system with βarr2, truncated at residue 382 and fused N-terminally to the large part of NanoLuc. These new CB1 and CB2 bioassays detected cannabinoid receptor activation by authentic serum or plasma extracts, in which synthetic cannabinoids (such as MDMB-CHMICA, AB-CHMINACA, 5F-PB-22, 5F-ADB, 5F-APINACA, EG-018, PB-22 and/or ADB-FUBINACA) were present at low- or sub-ng/ml level or in which Δ^9-tetrahydrocannabinol was present at concentrations above 12 ng/ml. For synthetic cannabinoid detection, analytical sensitivity was 82%, with an analytical specificity of 100%. **Conclusion:** The new CB1 and CB2 bioassays have the potential to serve as a first-line screening tool for (synthetic) cannabinoid activity in serum or plasma and may complement conventional analytical assays and/or precede analytical (mass spectrometry based) confirmation.

B-441

Therapeutic Drug Monitoring of Monoclonal Antibody in Inflammatory Bowel Diseases: Laboratory Evidence to Predict Patient Responses

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Background: Monoclonal antibody (mAb) biologic drugs that target inflammatory mediators hold promise in the treatment of inflammatory bowel disease (IBD). Therapeutic drug monitoring (TDM) of both drugs and anti-drug antibodies (ADAbs) is a valuable tool that can guide a personalized treatment plan. This study aims to provide laboratory evidence to predict patient responses to these therapies in IBD by reviewing TDM testing results of 6 biologics; adalimumab (ADA), certolizumab (CER), golimumab (GOL), infliximab (INF), ustekinumab (UST), and vedolizumab (VED). **Methods:** A total of 18,837 sera samples collected at trough levels from adult and pediatric IBD patients receiving mAb treatments were analyzed using the InformTx™ assay (Inform Diagnostics, Inc.), which employs an ELISA-based method to measure concentrations of drugs and free ADAbs. Patient responses were predicted on the basis of drug and ADAbs status. The needs for potential drug optimization were assessed by comparing drug and ADAbs concentrations with regard to the recommended therapeutic drug levels (ADA: 5.0-12.0 µg/mL, CER: >27.5 µg/mL, GOL: >1.4 µg/mL, INF: 5.0-1.0 µg/mL, UST: >4 µg/mL, and VED: >10 µg/mL) and laboratory-defined higher ADAbs levels (A-ADA: >25 ng/mL, A-CER: >25 AU/mL, A-GOL: 10.0 ng/mL, A-INF: >25 ng/mL, A-UST: >20 AU/mL, A-VED: 100 ng/mL). **Results:** 64.1%, 30.2%, 83.9%, 60.4%, 25.2%, and 69.1% of the patients treated with ADA, CER, GOL, INF, UST, and VED, respectively, had drug level equal to or greater than the recommended therapeutic level and undetectable ADAbs. 4.5%-33% patients had a drug concentration above the recommended therapeutic level. In contrast, patients (31.0% in ADA, 57.0% in CER, 12.1% in GOL, 32.5% in INF, 74.4% in UST, and 30.6% in VED) had undetectable or suboptimal levels of drugs and undetectable or lower levels of ADAbs. The overall ADAbs positive ratio for ADA, CER, GOL, INF, UST, and VED was 5.3%, 15.1%, 5.6%, 8.0%, 0.6%, and 0.4%, respectively. **Conclusion:** This study provides laboratory evidence to dictate the patient responses to mAb treatments in IBD patients. Undetectable or suboptimal drug levels may portend loss of response or unsatisfactory response to mAb therapies. Additional ADAb measurements are useful in distinguishing patients with low/undetectable levels of ADAbs (12.1% - 74.4%) who may benefit from dose escalation or shortening of dose interval from those with higher levels of ADAbs (0.3% - 12.6%) who need to be switched to different drugs. In patients with drug concentrations above the recommended therapeutic level (4.5% - 33%), de-escalation of therapy might reduce associated risks and costs. Moreover, the immunoresponse to mAbs varies among drugs with the lowest in UST and VED and is consistent with the degree of humanization of mAbs. Not surprisingly, the overall ADAbs positive ratios reported in this study were significantly lower than what have been previously described using methodologies that detect both free and drug-bounded ADAbs. Given that only the free ADAbs have the capacity to neutralize drugs in future infusions, quantitation of free ADAbs may represent a more clinically informative measurement. Additional clinical validation is imperative to confirm potential of TDM to improve efficacy, safety, and cost-effectiveness of these biologic therapies.

B-442

Stability of Oxidants in Urine Specimens Used for Specimen Validity Testing

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Background: The analysis of a urine specimen to determine if it is "consistent with normal human urine" is referred to as specimen validity testing (SVT). The measured indices normally include pH, specific gravity, nitrites, chromate, and oxidants. These indices are used to determine if a urine specimen has been diluted, adulterated, or substituted. The purpose of this study was to determine the stability of a urine specimen that has been adulterated using sodium hypochlorite (NaOCl) as an oxidant. **Method:** Two pools were created by combining patient urine specimens that had previously been tested negative for sample adulteration. Pools were spiked with NaOCl in order to achieve an initial oxidant concentration that would reflect results of "normal" (<200mg/L) and "adulterated" (≥200mg/L) as defined in the *Mandatory Guidelines for Federal Workplace Drug Testing.* The "normal" pool (8.0mL) was spiked with 10 µl of NaOCl and the "adulterated" pool (8.0mL) was spiked with 17 µl of NaOCl. Each pool was then tested for oxidants immediately after the spiking (time 0). The two pools were then aliquoted and stored at 4°C and -20°C for the following time periods: 0, 5, 10, 24, and 48 hours. Samples at each time point for each storage condition were assayed in triplicate by the Roche ONLINE DAT Specimen Validity Test Oxidant assay on the cobas c501 clinical chemistry analyzer. The mean results for each specific storage condition and time point were then compared to their respective time 0 mean. **Results:** The percent differences measured at each time interval for both pools were >20% in comparison to time 0 means and continuously decrease over the study course. **Conclusion:** Patient urine samples that are tested for the presence of adulterants by the SVT oxidant assay are not stable when NaOCl is used as the adulterant. This indicates that false negative results for oxidants may occur if urine samples cannot be tested immediately post collection.

B-443

Evaluation of Roche ONLINE TDM Acetaminophen Gen.2 assay and its robustness for analysis of hemolyzed samples

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Background and objectives: Sunnybrook Health Sciences Centre is a regional trauma centre with the largest Trauma, Emergency and Critical Care Program in Canada. Unfortunately, the currently used acetaminophen method (Roche Acetaminophen assay on MODULAR® P analyzer, Gen.1) is susceptible to hemolysis interference, resulting in high rates of sample rejection and turn-around-time delay in our patient population. This is especially challenging in our critical patients where samples can be more difficult to obtain. The objective of this study was to evaluate the performance of the newer generation Roche ONLINE TDM Acetaminophen Gen.2 assay (ACET2, on the Roche COBAS® c502 analyzer, Gen.2) and to confirm its robustness in the presence of hemolysis. **Methods:** The Gen.1 assay is an enzymatic method based on the hydrolysis of acetaminophen to p-aminophenol and acetate via arylacylamidase, with subsequent conversion of p-aminophenol to a chromogenic indophenol in the presence of o-cresol and sodium periodate. The Gen.2 assay is a homogeneous enzyme immunoassay based on competition between endogenous drug and G6PDH-labeled drug for anti-acetaminophen antibody binding sites. The Gen.2 assay was evaluated for its precision, linearity and accuracy based on CLSI guidelines. The hemolysis interference study was performed by spiking serum samples with a concentrated hemolysate stock solution. **Results:** Precision was assessed using two levels of Bio-Rad Liquichek IA Plus™ quality control material. The overall %CVs were 2.5% and 3.0% for the low (mean = 214 µmol/L) and high (mean = 657 µmol/L) quality controls, respectively, across 37 runs. Linearity was assessed using both TDM1 ACTM linearity material (Maine Standards) and patient samples. Across a concentration range of 38 to 3452 µmol/L (extended range with dilution), the assay demonstrated linearity with <10% difference from targets. Correlation between the Gen.1 and Gen.2 was assessed using patient samples with acetaminophen concentrations ranging from 36 to 687 µmol/L. Overall, the Gen.2 assay displayed an average negative bias of 21.7%

(n = 31) comparing with Gen.1. Due to this observed negative bias, accuracy of the Gen.2 assay was further assessed using external quality assurance (EQA) materials. The Gen.2 assay results were well within acceptable ranges when compared to the all methods mean. Finally, interference of hemolysis was assessed by spiking various levels of hemolysate into acetaminophen-naïve samples and samples with approximately 200 µmol/L of acetaminophen. Across a range of hemolysis levels (hemoglobin concentration 0 to 10 g/L), there was negligible interference in the Gen.2 assay for both acetaminophen-naïve and acetaminophen of 200 µmol/L samples (difference <6.2%). However, the Gen.1 assay gave various levels of false positive results in acetaminophen-naïve samples as well as falsely elevated results in the samples with acetaminophen. **Conclusions:** Evaluation of the Roche Gen.2 acetaminophen assay has shown that this immuno-based assay has an acceptable analytical performance and is less susceptible to hemolysis interference compared to the Gen.1 enzymatic method. Implementing the Gen.2 assay on our COBAS® c502 analyzer will thus allow for a lower sample rejection rate due to hemolysis and improve the quality of care, especially for our trauma, emergency and critical care patients.

B-444

Urine buprenorphine and metabolite patterns in a large cohort of patients

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Background: Products co-formulated with buprenorphine (BUP) and naloxone (NX), such as Suboxone and Zubsolv, are frequently prescribed as therapy for opioid use disorder. An integral part of therapy is monitoring for adherence. Understanding the typical patterns of BUP and metabolites in urine would inform interpretation of results. BUP metabolites include norbuprenorphine (NOR) and the glucuronide forms (B3G and N3G). Additionally, NX is measurable in urine. Our lab previously reported that 91% (n=1,946) had three (NOR/B3G/N3G) or four (BUP/NOR/B3G/N3G) metabolites present and that free BUP and NX concentrations >100 ng/mL were suggestive of adulteration. Our objective was to evaluate urine BUP and metabolites and NX in a recent, large cohort of samples and to assess the ratio of NOR+N3G to BUP+B3G (N:B) as a potential indicator of consistent therapy. **Methods:** Data for all quantitative urine BUP and metabolite tests performed by LC-MS/MS from February 2011 to January 2018 were retrieved. Cutoff concentrations were 2 ng/mL for free BUP and NOR, 5 ng/mL for B3G and N3G, and 100 ng/mL for NX. Results were analyzed for: 1) the occurrence of BUP and metabolites; 2) the distribution of concentrations for NX, BUP and metabolites and; 3) the ratio (%) of N:B in patients with at least 50 separate test requests for comparison. **Results:** Results for 128,709 tests requests were obtained from 44,299 different patients. The median age was 34 years (interquartile range (IQR) 27-44 years). Similar to previous, 93.5% of patients had three or four metabolites present (NOR/B3G/N3G=40.6%; BUP/ NOR/B3G/N3G=52.9%). Of the total cohort, 24.9% of samples were negative for all compounds. There were 3,342 (2.6%) samples with NX, of which 2,030 (60.7%) had both NX and BUP concentrations ≥1000 ng/mL suggestive of sample adulteration. There were 912 patients with BUP >100 ng/mL but <1000 ng/mL. Within those 912 patients, 162 had no quantifiable metabolites (including 62 with NX <100 ng/mL), while 631 patients had quantifiable metabolites and NX <100 ng/mL, suggestive of possible adulteration with a BUP product not co-formulated with NX. Among 58 patients with at least 50 incidences of monitoring, the ratios of N:B varied significantly inter-individually ($p<0.0001$) and intra-individually (mean CV%=55.2±17.3%), likely indicating variation in urine collection time since last dose, changes in dose, or adulteration. However, the N:B ratio was significantly different among three groups representative of samples from patients who had not recently taken the medication (late metabolism), had recently taken the medication (mid-metabolism), and those with ≥1 analyte ≥1000 ng/mL (early metabolism/high-dose therapy/adulterated sample) (median (IQR): 343 (243-543) ng/mL vs. 271 (159-452) ng/mL vs. 169 (129-251) ng/mL, respectively, $p<0.0001$). **Conclusion:** More than 90% of patients taking a BUP formulation will have three or more metabolites present in their urine. Which metabolites are present and the N:B ratio may aid in assessing the stage of metabolism or if adulteration has occurred. Consistency with respect to the time of dose, time of urine collection, and consideration of urine dilution may improve the utility of metabolite ratios to evaluate whether a patient is being adherent with their therapy.

Wednesday, August 1, 2018

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

Technology/Design Development

B-445

Analytical evaluation and clinical performance of an enzyme-linked immunosorbent assay for measurement of afamin in human urine

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Background: Afamin is the fourth member of the albumin superfamily, which comprises albumin, α-fetoprotein and vitamin D-binding protein. Although the presence of urine afamin has been verified by mass spectrometry and western blot, there is still a lack of a robust enzyme-linked immunosorbent assay (ELISA) method for determination of urine afamin (uAFM). The aim of this study was to evaluate the analytical characterization and clinical value of an ELISA for measurement of uAFM. ***Methods:*** We determined uAFM and calculated afamin-creatinine ratio (AfCR) of 136 healthy volunteers and 129 biopsy-proven glomerulonephritis patients. We evaluated precision, linearity and detection limit of the assay and determined reference intervals according to the Clinical and Laboratory Standards Institute (CLSI) guideline. ***Results:*** The percentage coefficients of variation of repeatability and within-laboratory precision were 12.2% and 12.5% at a mean concentration of 38.85 ng/mL, and 5.4% and 14.0% at a mean concentration of 12.47 ng/mL. Linear range of the method is 1.95-76.41 ng/mL. The limit of blank and the limit of detection were 2.31 ng/mL and 3.21 ng/mL. uAFM and AfCR values were different significantly between males and females. For uAFM, the reference intervals were < 65.60 ng/mL (males) and < 37.20 ng/mL (females). For AfCR, the reference intervals were < 75.26 μg/g (males) and < 47.75 μg/g (females). In the clinical performance evaluation, uAFM and AfCR levels were significantly increased in patients with PMN, IgAN and MCD. uAFM and AfCR were positively correlated with urine albumin and albumin-creatinine ratio, respectively, rather than eGFR. ***Conclusion:*** Our study provided supports that the assay is a reliable and robust test for measuring uAFM. uAFM and AfCR may be attractive biomarkers for glomerular barrier function.

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Comparing operational performance of available immunochemistry systems using six different workloads

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Objective:
Laboratories face the pressure to deliver quality results as fast as possible, thus time to result is a key performance indicator of today's laboratories. In our study we compared the operational performance of five commercially available immunochemistry systems while processing different types of workloads under standardized conditions:
Methods:
Two workloads with request patterns reflecting a commercial laboratory and a hospital laboratory setting (each for 100 samples), were processed in the same manner on the following five immunochemistry systems: ADVIA Centaur XPT, ARCHITECT i2000SR, **cobas** 8000 e 801, Immulite 2000XP*i* and UniCel DxI 800. The 100 samples of the commercial laboratory workload resulted in 176 test requests. The 100 samples of the hospital laboratory workload resulted in 135 requests including 8 emergency samples. Furthermore, four infectious disease panels were measured with 50 samples (using negative control material) on the following four immunochemistry systems: ADVIA Centaur XPT, ARCHITECT i2000SR, **cobas** 8000 **e** 801 and Liaison XL. Panel 1 (pregnancy) consisted of HBsAg, HIV, Toxoplasmosis IgG, Toxoplasmosis IgM, Rubella and Syphilis; Panel 2 (Hepatitis/HIV) consisted of anti-HAV, anti-HAV IgM, anti-HBc, anti-HCV, HBsAg and HIV; Panel 3 (blood screening 1) consisted of HIV, HBsAg, anti-HCV, Syphilis and anti-HBc. Panel 4 (blood screening 2) consisted of HIV, HBsAg, anti-HCV and Syphilis. Time to first result and time to last result were reported. Starting point was the loading of the samples onto the analyser.
Results:
The hospital workload was processed within ~1h on two (**cobas e** 801 and ADVIA Centaur XPT) of the five applied analysers, maximum duration was 2:52h on Immulite 2000XP*i*. Similarly, processing time for the commercial pattern workload ranged from ~1h to ~2.5h. Across the four varying serology panels processed on four different analysers, time to first result was ≤21 min on the **cobas e** 801 module, ≤30 min on the ARCHITECT i2000SR, ≤32 min on the ADVIA Centaur and ≤53 min on the Liaison XL. Time to last result ranged from ≤02:38 h (**cobas e** 801 module) to 04:25 h (Liaison XL).
Conclusions:
During this study, the time to result differed considerably between the included immunochemistry systems. Differences up to more than 100% in operational performance, depending on the panel, may be seen when comparing identical workloads on the six tested immunochemistry systems.

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A Comparison Study of qPCR and ddPCR Assays in Measuring Plasma Epstein-Barr Virus DNA Levels

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Background: Plasma Epstein-Barr Virus (EBV) DNA is a routine test in molecular diagnosis laboratory, for confirming EBV infection, to evaluate therapeutic efficacy in patients taking immunosuppressant with autoimmune disease or after transplantation, and to aid in lymphoma and nasopharynx cancer diagnosis and prognosis. However, hyposensitivity has been always criticized in clinical application. Development of digital PCR exerts enormous potential in molecular diagnosis owing to its high sensitivity and its ability of absolute quantification. This study was conducted to compare the droplet digital PCR(ddPCR) and routine qPCR method for detecting EB viral load. **Methods:** A total of 510 patients (immunocompromised:201; lymphoma:128; untreated nasopharyngeal carcinoma:39; treated nasopharyngeal carcinoma:142) who were highly suspected with EB infection were enrolled in the study, DNA was extracted from Plasma, BamHI-W fragment was amplified by qPCR using EB viral load quantification kit (Sansure Biotech), while ddPCR was performed by BIO-RAD QX200. **Results:** Based on ddPCR method, 369 patients were EBV positive, the median of viral load was 360 copies/mL(P_{25}-P_{75}:67-2905copies/mL). Among the four patients groups, EB viral load of untreated nasopharyngeal carcinoma patients was highest, the median viral load was 4590 copies/mL, followed by lymphoma (840copies/mL) and treated nasopharyngeal carcinoma patients (430 copies/mL), and the immunocompromised patients (130copies/mL) was the lowest. Since the cutoff point of qPCR was 400 copies/mL(designated by the kit), most EBV positive patients (252/369) were missed by qPCR. Hence we reviewed all of the amplification curves of qPCR, 231 of 252 false-negative EBV by qPCR had typical amplification curves, if we designated those with typical curves as positive, qPCR sensitivity would improve greatly, nevertheless the false-positive EBV also raised (53 patients). We attempted to perform ROC analysis, setting the cutoff value as 10.6 copies/mL showed the best diagnostic efficacy in our data. On the other hand, when EBV were detected positive by both the two methods, the quantitative values of EB viral load were moderately accordant(R^2=0.533). **Conclusion:** EBV viral load is mostly very low in clinical practice, routine qPCR method is difficult to satisfy clinical demand, optimize qPCR to increase its sensitivity or replace it with ddPCR is a considerable choice.

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Evaluation of methods for the collection and enrichment of mRNA in liquid biopsy samples

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Background: Liquid biopsy is a low invasive procedure which can be repeatedly conducted, making it suitable for long-term monitoring of disease. Circulating RNAs in liquid biopsy are protected by extracellular vesicles (EVs). Among them, messenger RNAs (mRNAs) are promising markers in EVs. However, most previous studies focused on exosomes and their containing microRNA markers. Only few reports addressed the issues of mRNA collection. The aim of this study is to compare different methods for the collection and

enrichment of EVs and containing mRNA markers in liquid biopsy samples. **Methods:** Conditioned medium from *in vitro* culture, EDTA-plasma, and serum were used as liquid samples. We first applied sequential centrifugation and ultra-centrifugation to separate larger particles (mainly microvesicles and apoptotic bodies) from smaller particles (mainly exosomes), and examined the distribution of mRNA. Then we tested the efficiency of two methods to enrich EVs, including ExoEasy kit which bound all membrane vesicles, and magnetic beads which captured vesicles having phophatidylserine. The collected EVs were subjected to RNA extraction and the purified mRNAs were quantitated by real-time reverse-transcription PCR for housekeeping gene transcripts. **Results:** We could purify and amplify mRNA from these liquid samples. In the centrifugation experiments, we found that the majority of mRNAs were associated with larger particles. In the enrichment experiments, magnetic beads that bound to phophatidylserine had a higher efficiency of enriching and collecting mRNA than the ExoEasy kit. In addition, the amount of mRNA recovered from serum was slightly higher than that from plasma. **Conclusion:** This study demonstrates that circulating mRNA is mostly encapsulated in larger particles, probably microvesicles or apoptotic bodies. To collect these particles, centrifugation at high speed should be avoided, and magnetic beads is helpful for enrichment. The optimized procedures will be applied to analyze cancer-specific mRNA markers in our future studies.

B-449

The diagnostic accuracy of Xpert MTB/RIF for pulmonary tuberculosis: A systematic review and meta-analysis.

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Background: Pulmonary tuberculosis accounts for 80% of all kinds of tuberculosis which is the ninth leading cause of death in the world. Xpert MTB/RIF is a novel diagnostic tool for pulmonary tuberculosis, however, its diagnostic performance has not yet been reached consensus. The objectives of this study were to evaluate the diagnostic accuracy of Xpert MTB/RIF referenced to culture and provide some reliable advice for clinical practice. **Methods:** A comprehensive literature search of Web of Science, PubMed and Embase was conducted from their reception to October 9, 2017. Data from included studies were pooled to yield summary sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) and area under the curve (AUC) with a 95% confidence interval (95% CI) to determine the diagnostic performance of Xpert MTB/RIF. The bivariate random-effects model was carried out in quantitative synthesis. Quality assessment was performed according to each question of the Revised Tool for Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). All statistical analysis was performed by Meta-DiSc software v.1.4 and Review Manager V.5.3. **Results:** Thirty-three studies were included in our analysis with a total of 19768 participants. The pooled sensitivity and specificity were 87.2% (95% CI: 0.861-0.882) and 96.6% (95% CI: 0.962-0.969), respectively. The AUC of Xpert MTB/RIF was 0.973. The heterogeneity of all articles could be accepted. **Conclusion:** According to our research based on a more strict definition of pulmonary tuberculosis, it is a better choice to apply Xpert MTB/RIF to diagnose this disease regarding its high specificity. Although its sensitivity may be lower than culture, it can provide a result within a shorter time and is more suitable for rapid diagnosis and prompt treatment.

B-450

BAMEditor : a benchmarking toolkit for somatic variant detection

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Background:
The molecular diagnostics of cancer by the adoption of next-generation sequencing (NGS) are transforming to match identified genetic alterations with clinical actionable strategies. However, the complexity of the human genome sequence and NGS methods makes the variant detection challenging, especially for somatic variants detection of the target sequencing of cancer. Several studies have indicated that NGS bioinformatics pipelines with different variant calling algorithms and parameters exhibit substantial discrepancies among variant calls. Without a uniform gold standard, clinical laboratories may generate hidden, and/or inaccurate results due to improperly developed, validated, and monitored bioinformatics pipelines. Although many cancer genome simulation tools have been made available, they often cannot fully model the sequencing errors and other sources of error introduced during target capture and library preparation or exist some limitations. Therefore, to create a comprehensive gold standard for somatic mutation detection, we developed a cancer genome simulator for somatic mutation detection. **Methods and Results:** Here we present the BAMEditor tool, a newly developed tool for mutation simulation by editing the existing reads. BAMEditor can add a comprehensive of mutations, including single-nucleotide variant (SNV), small insertion and deletion (Indel), copy number variation (CNV) and large structural rearrangement to any alignment stored in BAM format, such as whole genome, whole exome and targeted sequence data. In addition, BAMEditor also supports flow space data in Ion Torrent sequencing reads. As a demonstration of the utility of BAMEditor, we invited 125 clinical laboratories and academic medical centers performing NGS routinely to process our synthetic tumor-normal pairs by applying BAMEditor to an already sequenced cell line. In this survey, our analysis reveals the contributions of individual pipeline components and parameters on the accuracy of variant detection. We found that variant calling algorithms and variant filter strategy are the key point of variant detection.
Conclusion:
In summary, our BAMEditor tool provides a comprehensive resource for somatic variant detection benchmarking. In addition, our survey provides a comprehensive assessment of bioinformatics pipelines for variant detection of target sequencing which may propose useful guidelines for the benchmarking of bioinformatics pipelines.

B-451

Increased Resilience of Aspiration Monitoring, Incorporation of Commodity Dilution and Interface with Existing Laboratory Infrastructure in the Alinity i System

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Background: Advanced technologies have enabled improvements in instrument autonomy, reliability and electromagnetic compatibility.
• Wash monitoring [WM] can detect errors such as loose connections, decreased vacuum and improperly prepared samples real-time as well as predict future malfunction. Optimized instrument functionality and algorithm enhancements also contribute to decreased false sample/reagent detection.
• Wash buffer is a common medium for immunoassay analyzers in the field and is typically reconstituted in a stand-alone system, with carboys being transported to the immunoassay analyzer by laboratory personnel. Incorporating the reconstitution of wash buffer into the instrument would allow onboard dilution on demand.
• High volume laboratories often rely on a track system to distribute patient samples to the different instruments available in their labs. Incorporating next generation analyzers with these track systems and lab informatics software will allow progression of detection technologies via adoption by established laboratories.
Methods:
• Inaccurate liquid detections from agents such as bubbles or unintended contact between the probe and the vessel wall were mitigated by adding enhancements to the WM algorithms to detect and filter out such events. Using embedded capacitance and ferrites tuned to be resistive at certain frequencies, WM has reduced its noise footprint and become less susceptible to external electromagnetic interference.
• Technological development of an embedded system to supply the instrument with wash buffer on demand was incorporated into immunoassay analyzers. Sensors monitor functions to ensure the dilution is being made as intended and careful material selection promotes the same quality offered by a stand-alone system.
• Design considerations focused on interfacing both software and hardware with the existing track systems to encourage integration while still allowing operators to override the track scheduling with STAT samples. Automation and Informatics reduce the human interaction with the samples and information to improve turn-around time, reduce human error and distribute the patient load across instruments while ensuring optimum handling conditions.
Results:
• The enhancements and optimizations to WM increased the resilience and robustness of the measurements by improving the reliability of accurate liquid detection to 99.8%. Incorporation of the onboard reconstitution station has resulted in a reduction of footprint and increased walk-away time for laboratory personnel.
• Technicians spent up to 57 minutes replenishing supplies on previous systems, but with the system generating wash buffer on demand, that replenishing time is cut to zero. Previous generations required the use of a separate reconstitution station at a cost of 2.2 square meters of laboratory real estate. The incorporation of the station into the instrument means that separate footage cost is cut to zero and due to other technological advances, the instrument is still half the size of the average previous generation system.
• Next generation analyzers interface with the track while still allowing access to the processing bay for operators to run STAT samples. Software is universal across the family of instruments and interfaces with existing lab informatics software to ensure continuity despite adoption of new technology.

Conclusion: Improvements in autonomy, reliability and electromagnetic compatibility have been realized in the next generation analyzer technologies.

B-452

2018 AACC Carryover Reduction

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Introduction
Sample carryover is an inherent risk and can cause erroneously high patient results for immunoassay tests. In the IVD industry, sample to sample carryover has been the main focus and has been tested over various systems but there are other areas in which carryover can occur. Sample to sample (= external carryover) and total system carryover (= internal carryover) must be assessed to secure patient results.
Method
As part of new system development, three areas were evaluated to minimize carryover; a) sample pipettor for aliquots, b) sample pipetter for tests, and c) first aspiration probe in bound/free separation process. 1. To eliminate sample carryover, the new system uses a single-use pipetting tip for a) each aliquot sampling and for b) each test sampling. 2. Amount of probe carryover is tiny and below assay detection limit, then direct Alkaline-phosphatase (= ALP) reaction with substrate was tested to evaluate first aspiration probe carryover. Add 500µL of high concentrated Alkaline-phosphatase (4.2 µg/mL) into a reaction vessel and measure relative light unit (= RLU) of following known negative buffer. 3. To evaluate total system carryover, three known high positive HBsAg samples and known negative samples were tested together using Access HBsAg test kit.
Results
1. The engineering design solution of single-use pipetting tips provide 0 carryover 2. Observed RLU was 7,959 which corresponds to 2.865 ppm. The protocol uses 21 times high ALP concentration and 3.333 times larger reaction volume than Access2 HBsAg test kit (25µL of 1.0 µg/mL conc. of ALP into 150µL of total reaction volume), therefore possible maximum HBsAg carryover can be 0.0409 ppm (= 2.865/21/3.3) 3. Observed HBsAg carryover results on negative samples were 0.0*** ppm, 0.0*** ppm, below the assay detection limit.
Conclusion
The new system with single-use pipetting tip eliminates sample to sample carryover and demonstrates <0.1 ppm or below the assay detection limit for total system carryover to prevent false positive results.

B-453

Validation of high sensitive troponin T in Roche cobas 8000 system

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Background: Following the FDA approval, the long-awaited high sensitivity troponin is finally available in the US. The Roche Troponin T Gen 5 STAT assay is a highly sensitive and specific marker for myocardial damage. Cardiac troponin increases rapidly (as early as within 1 hour detected by high sensitivity assay) after acute myocardial infarction and may persist for up to 2 weeks. This new assay identifies and measures cardiac troponin at previously undetectable levels, enabling earlier diagnosis of acute coronary syndrome (ACS), faster rule-out of acute myocardial infarction (AMI), and improved prediction of adverse outcomes. The performance of the Troponin T assay meets the requirements of The Third Universal Definition of Myocardial Infarction (MI) and the IFCC recommendations. The assay performance was validated following the requirements of regulatory agencies and CLSI guidelines as part of the process of implementation. **Methods**: The within-run and between-run precision was assessed at five levels by measuring Bio-Rad, Roche PreciTroponin and Randox Cardiac quality control materials following CLSI guidelines EP15-A3 and EP6-A. Linearity and accuracy was assessed using Roche Calcheck material. Interference from known unconjugated bilirubin, triglycerides and hemoglobin materials (Sun Diagnostics, LLC, New Gloucester, ME) was assessed. Comparison between Roche Troponin Gen 5 STAT and TnI assay on Beckman DXI or cardiac troponin T (TnT Gen4) on Roche was conducted. **Results**: The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) of the hs-TnT assay were 2.5, 3, 6 ng/L, respectively. The linearity was in the range from 0 to 9850 ng/L. Linearity verification revealed slope=1.026, y-intercept=0.00. A five-day precision study assayed in duplicate at two separate times of the day demonstrated within-run precision of CV<7%, between-run of CV <10%. Interference studies revealed no significant interference of bilirubin, triglycerides and hemoglobin on troponin levels. The comparison study was analyzed using Deming regression. With the concentrations of TnI from 0 to 2.09 ng/mL, the slope, y-intercept and correlation coefficient (r) was 470, 3.58, 0.978, respectively. There is a good correlation with troponin T Gen 4 with the slope, y-intercept and correlation coefficient (r) was 1004, 3.14, 0.995, respectively. The reference intervals were verified and established with the cutoff at 19 ng/mL for both males and females. **Conclusion**: In conclusion, The Roche cobas 8000 system is a robust, high-throughput method for TnT. The performance of the Troponin Gen 5 assay is acceptable for patient testing in clinical laboratories.

B-454

The New Total Bile Acids Assay for the Architect cSystems Instrument

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OBJECTIVE: To present the performance and interference test results of the new Abbott ARCHITECT Total Bile Acids assay on the *c*Systems instruments. **RELEVANCE:** The new Total Bile Acids assay (list number [LN] 03R04) is now liquid, ready-to-use, with longer on-board and calibration intervals, which can be used to measure both plasma and serum bile acids. It utilizes a decreased sample volume relative to the predicate Bile Acids LN 06K90 assay. **METHODOLOGY:** The Total Bile Acids assay (LN 03R04) utilizes the enzymatic cycling colorimetric methodology. The enzyme, 3-α-hydroxysteroid dehydrogenase, reversibly oxidizes the bile acids in the sample to their respective 3-α-oxosteroids in the presence of excess nicotinamide adenine dinucleotide (NADH) and thionicotinamide adenine dinucleotide (thio-NAD^+). During this reaction, thio-NAD^+ is reduced to thio-NADH. The rate of production of thio-NADH is monitored at 404 nm and is proportional to the concentration of bile acids in the specimen. **VALIDATION:** The table below displays the performance characteristics of the new Total Bile Acids assays (LN 03R04) relative to the predicate assay LN 06K90. The interference results for the new Total Bile Acids 3R04 is displayed in the lower portion of the table. All interference data shown, represent the highest acceptable interference levels.

Characteristic	LN 06K90				LN 03R04			
Configuration	Lyophilized				Liquid, Ready-to-Use			
Sample Type	Serum				Serum and plasma			
Sample Volume (µL)	32.0				3.0			
Imprecision	N	Mean (µmol/L)	Total SD (µmol/L)	Total %CV	N	Mean (µmol/L)	Total SD (µmol/L)	Total %CV
	48	3.08	0.21	6.95	80	3.3	0.2	5.1
	48	12.69	0.30	2.40	80	9.7	0.2	1.9
	48	44.03	0.49	1.12	80	18.0	0.3	1.7
	-	-	-	-	80	48.0	0.9	1.8
	-	-	-	-	80	167.3	2.8	1.7
Method Comparison:	Abbott AEROSET vs. Comparative Method				03R04 vs. 06K90			
	N	62			N	136		
	R	0.9906			R	0.997		
	Equation	Y = 0.9659x – 0.4914			Equation	[03R04] = 1.08[06K90] – 1.44		
	Range (µmol/L)	3.3 – 49.7			Range (µmol/L)	1.4 – 148.9		
On-Board and Calibration Stability in hours	168 (7 days)				672 (28 days)			

Interferent	[Interferent]	[Bile Acid] (µmol/L)	Difference (µmol/L)	% Difference
Bilirubin (Conjugated)	22.4 mg/dL	4.9	0.1	2.8
	22.4 mg/dL	21.7	0.4	1.9
Bilirubin (Unconjugated)	14.9 mg/dL	5.0	0.6	11.2
	29.9 mg/dL	22.0	1.5	7.0
Hemoglobin	1000 mg/dL	2.9	-0.5	-15.9
	1000 mg/dL	23.1	-1.5	-6.4
Human Triglycerides	1739 mg/dL	4.7	0.2	4.7
	1703 mg/dL	20.7	-0.2	-0.8
Intralipid	750 mg/dL	4.9	0.5	10.4
	2000 mg/dL	19.7	1.5	7.7
Protein	14.0 g/dL	5.2	-0.5	-10.4
	12.2 g/dL	24.7	-2.5	-10.1

CONCLUSIONS: The new Total Bile Acids LN 03R04 assay displays enhanced performance characteristics and improved ease of use over the predicate Bile Acids LN 6K90 assay.

B-455

Kinetics Study of Hemolysis: Evaluation of the Hemolytic Strength of Lytic Reagents

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Background:
Cell lysing solutions are essential reagents used by hematology analyzers to categorize and enumerate cell types such as erythrocytes (RBC), thrombocytes (platelets), and leukocytes (WBC) in whole blood. Lysing reagents are not easily pre-assessed for strength before formulation and use in hematology assays. Lytic strength depends on the chemical properties and the concentration of the lytic agent. The relationship between the lytic strength and the hemolysis rate has been mostly an experimental practice in laboratories and hasn't been well characterized. We propose to study the lysis kinetics of red blood cell control samples by lytic agents or commercial lysing reagents for assessing their hemolytic strength, since it is directly related to the determination of the hemolysis rate constant. The study of the lysis kinetics of control samples by the lytic agent, sodium dodecyl sulfate, was to verify our hypothesis: whether the reaction followed first order kinetics and the possibility of using the lysis rate constant to assess the hemolytic strength of the lysing reagents.
Methods:
The kinetics study of the hemolysis was conducted with a control blood sample and a lytic agent: sodium dodecyl sulfate (SDS). The course of hemolysis was monitored on a UV-Vis spectrophotometer, which measured absorption. The absorption measure comes from RBC particles scattering incident light, which decreases as the number of particles diminishes. The lysate was monitored at a wavelength of 700 nm to avoid potential interference from hemoglobin absorption. Concentration of RBC was fixed and lytic agent concentrations were varied in the experiment.
Results:
Lysis of the blood control sample by the lytic agent yielded S-curves of the time course of absorption change due to light scattering by RBC particles. The A_{half} point was the point at which the absorbance value reached half of the initial absorbance + the background absorbance. The time at which this value was reached was recorded as T_{half}. Analysis of the data of variables $1/T_{half}$ and lyse concentration demonstrated a linear relationship, which confirmed the first order kinetics of hemolysis. The lysis rate constant is obtained by the slope of the linear regression equation.
Conclusion:
We have demonstrated that $1/T_{half}$ of the cell lysis reaction has a linear relationship with the concentration of the lytic agent, SDS. We confirmed the hypothesis that the RBC lysis followed a first order reaction kinetics given a constant and excessive concentration of SDS. Hemolytic potential can be extracted from the slope of $1/T_{half}$ vs [Lys], which is 1.44k, k is the pseudo first order rate constant. We have potentially found a better way to assess the lytic strength of the lysis reagent for hematology assays.

B-456

Performance on site evaluation of the new Abbott Alinity i system

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Background: The goal of our study was to evaluate the analytical performance of Alinity i, Abbott's next-generation immunoassay (IA) system in independent laboratory setting. **Methods:** Alinity i is based on chemiluminescent microparticle immunoassay (CMIA) detection technology for the quantitative determination of analytes in human serum, plasma, urine and cerebrospinal fluid. Performance profiles including precision, linearity, limit of quantification and method comparison with our routine analytic platforms (Vista 1500, Siemens / IDS-iSys, Immunodiagnostic Systems / Architect i2000, Abbott) for selected assays (TSH, fT3, fT4, high sensitive cardiac troponin I (hsTnI), vitamin D 25) were assessed following the CLSI guidelines. **Results:** The observed total imprecision (CV) ranged from 1.54% to 5.42%; the recovery calculation for the linearity experiment showed reasonable values between 91.8% and 116.4%, except for fT3 (<80%); the correlation slope values (Passing-Bablok) varied from 0.88 to 1.11. We additionally report the limit of quantification for TSH in serum (0.0081 IU/l, CV 3.7%) and hsTnI in plasma (5.35 ng/l, CV 7.7%) as representative examples. **Conclusion:** Precision, bias and correlation to the current methods have been satisfying and reflected the manufacturer's declared performance. The fT3 assay showed a disturbed linearity. LOB, LOQ, LOD of the TSH and hsTnI assays exceeded the manufacturer declared performance showing high precision in the lower measuring range.

B-457

Induction Heating: An Advanced Decontamination Technology to Preserve Sample Integrity on Abbott ARCHITECT i2000SR and Alinity i Analyzers

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Background: In automated diagnostic testing, sample integrity can be at risk during sample pipetting steps due to the potential for sample contamination from a previous sample (carryover). Common industry practice is to use replaceable pipette tips (increasing cost to the user and disposable waste) or fluidic cleaning processes whereby mechanical and chemical mechanisms are used to decontaminate a re-usable pipette. Abbott platforms have proven, robust sample car-

ryover mitigation by leveraging patented technologies and designs with re-usable pipettes. Recent breakthroughs in assay sensitivity, however, require a more advanced decontamination technology to minimize risk of sample carryover and preserve sample integrity. To meet this need, we present a novel application of induction heating on the ARCHITECT i2000SR and Alinity i systems. **Methods:** A recently published method (US 9,073,094B2) discloses a novel contact-free decontamination technology whereby a metal pipette is inductively heated. With this technology, the pipette warms from its own resistance to coil-induced electrical currents (Joule heating). By sweeping the pipette through an induction coil, temperatures on the pipette are elevated throughout its length. To demonstrate this technology, a next-generation, high sensitivity HBsAg immunoassay was used to quantify carryover using the ARCHITECT i2000SR and Alinity i platforms. These platforms were upgraded with the induction heating technology and performed tests organized to induce carryover from sample cups into reaction vessels and between sample cups. **Results:** Tests yielded a quantified carryover ($\mu \pm \sigma$) on the ARCHITECT i2000SR and Alinity i of 0.002 (± 0.001 ppm) and 0.002 (± 0.002 ppm), respectively. Induction heating reduced carryover well below requirements supportive of next-generation, high sensitivity assays and more than 10x below the current requirement of 0.1 ppm. **Conclusion:** Results presented here underscore the effectiveness of this technology in controlling sample integrity, thereby reinforcing the accuracy and continued confidence of results from Abbott ARCHITECT i2000SR and Alinity i platforms. Furthermore, induction heating enables the development of higher sensitivity detection assays without having to sacrifice the benefits of using re-usable pipettes.

B-458

markBTM: A Novel Point-of-Care Immunoassay Platform for Quantification of Blood Biomarkers

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Background: To examine analytical performance of BBB's markB™ point-of-care immunoassay platform for the quantification of analytes in human blood. markB™ is based on BBB's novel power-free plasma isolation technology and magnetic electrochemical sandwich immunoassay, so-called MESIA. Once a drop of finger-prick blood is loaded on the markB™ strip, pure plasma is spontaneously separated from the whole blood by a filter membrane and flows through a microchannel to a reaction chamber by capillary action. The plasma dissolves pre-spotted detection probes, which are magnetic nanoparticles coated with electrochemical labels and target-specific antibodies. In MESIA, magnetic field drives efficient reactions among the detection probes, target analytes, and capture antibodies on the electrochemical sensor to form sandwich complexes. After the active mixing process, magnetic field removes unbound detection probes from the electrochemical sensor. Finally, the amount of analytes in the sample is quantified by measuring electrochemical signals from the probes bound on the sensor surface. markB™ platform was validated for a blood tumor marker, prostate-specific antigen (PSA). **Methods:** markB™ strip consists of two modules for power-free plasma isolation and identification of target analytes based on MESIA, respectively. A filter membrane for the complete removal of blood cells was embedded in a thermoplastic device having hydrophilic microchannels. The detection probes, 500-nm-diameter gold-coated magnetic nanoparticles conjugated with anti-PSA antibodies, were pre-spotted and dried in the reaction chamber. Screen-printed carbon electrodes were utilized for the electrochemical detection based on cyclic voltammetry. The electrochemical sensor was composed of three electrodes - the working, counter, and reference electrodes, and anti-PSA capture antibodies were immobilized on the working electrode. For analysis, a strip was placed on a mobile markB™ reader system, and ~ 30 µL of whole blood sample was loaded. Once the reaction chamber was filled with the blood plasma, the MESIA procedure was automatically performed by two permanent magnets, and the test result was obtained within 10 min. For preliminary evaluation of analytical performance of markB™ immunoassay system, more than 10 strips from 2 distinct lots were tested. **Results:** Once the blood sample was loaded on the system, the whole processes of plasma separation and immunoassay for quantification of PSA were automatically performed. The plasma was successfully separated from the whole blood without any external forces. According to the preliminary evaluation of analytical performance of markB™ for PSA, the lower and upper limit of detection of were determined to be 0.1 and 50 ng/ml, respectively. The mobile features of markB™ reader system enabled to measure, store, transfer, and manage the analysis records. **Conclusion:** markB™ allows for the quantification of target analytes in a tiny amount of whole blood sample without any moving elements or buffer exchange steps as well as without any bulky and expensive detection components. This technology would provide a solution for the needs of point-of-care testing market, which have sought a method for simple, automated, rapid, and accurate detection of disease markers from a drop of biological fluids using a handheld device.

B-459

Evaluation of the ASI Evolution® Automated RPR Syphilis Analyzer and the Repeatability of the Interpretation of Serological Syphilis Screening.

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Background:
The evaluation of the ASI Evolution Automated RPR Syphilis Analyzer to determine its ability to identify persons with possible syphilis infection using the CDC recommended nontreponemal algorithm[1]. A manual RPR test is dependent on good procedural technique with adequate lighting and visual acuity to interpret the presence of flocculation. A manual RPR test is subject to a lack of consistency and standardization between personnel interpreting results. To bring standardization and consistency to the interpretation of the RPR test, the ASI Evolution fully automated RPR analyzers was developed using a camera and mathematical algorithms to analyze the test well images and differentiate between flocculation and non-flocculation. To determine the consistency of the analyzer, a repeatability study was also performed to determine if there were any variations in measurements taken by the instrument on the same item and under the same conditions.
Evaluation - Methods and procedures:
The interpretation of 3757 serum and plasma specimens using the ASI Evolution were evaluated with the results by the ASiManager-AT. The testing requirements were as follows:
1. All samples were qualitative tested using the ASiManager-AT with cards manually prepared.

2. All samples were qualitative tested using the ASI Evolution automated syphilis analyzer.

3. The results of the two methods were evaluated for agreement.

Evaluation Results:
A total of 3757 serum and plasma specimens were evaluated to determine reactivity. Of the 3757 specimens, 1629 were reactive and 2128 specimens were nonreactive. The ASI Evolution identified 1629 of the 1629 reactive specimens as reactive and 2128 of the 2128 nonreactive specimens as nonreactive. A sensitivity of 100% and a specificity of 100% were determined. The reactive samples ranged in reactivity from minimal 1:1 titers to 1:64 titers.
Repeatability - Methods and procedures:
The interpretation of 10 specimens using the ASiManager-AT and the ASI Evolution were evaluated for reactivity. The testing requirements were as follows:
1. All qualitative testing was conducted using the procedure in the package insert.
2. Each qualitative sample was tested 192 times.
Repeatability Results:
A total of 10 specimens were evaluated to determine repeatability of reactivity. Of the 10 specimens, 7 were reactive and 3 were nonreactive. The reactive samples had titers of 1:1 (4 samples), 1:2 (1 sample), 1:8 (1 sample), and 1:256 (1 sample). Each of the 10 specimens were repeated 192 times to evaluate the reactivity. Results showed 100% concordance for each sample.
Conclusion:
The data above shows that the ASI Evolution gives an objective and standardized interpretation of the test results with a high degree of accuracy and repeatability.
References:
1. CDC, 2011. "Discordant Results from Reverse Sequence Syphilis Screening - Five Laboratories, Untied States, 2006-2010", *Morbidity and Mortality Weekly*, 60(05);133-137.

B-460

A basic performance of novel automated coagulation analyzer

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Background: We developed a new automated coagulation analyzer as successor instrument to STACIA™, that allows for high performance measurements despite the compact size. The features of this instrument are as follows. 1) A maximum throughput is achieved up to 240 test per hour for full random access. 2) A high on-board reagent stability is accomplished by using an automatic openable bottle top. 3) A risk of cross-contamination is avoided by employing a non-contact-stirring system. Here, we will report a basic performance of the new automated coagulation analyzer in detail. **Methods:** The new coagulation analyzer we developed demonstrates coagula-

tion time, latex photometric immunoassay (LPIA) and chromogenic assay as an assay principle. At first, we investigated a basic performance of the following assays: prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin III (AT III), fibrinogen/fibrin degradation products (FDP) and D-dimer on the new analyzer. Next, we also evaluated the other applicable reagents, fibrinogen, thrombotest, hepaplastintest, coagulation factor activity, thrombin-antithrombin III complex (TAT), soluble fibrin (SF), protein C (PC), factor XIII, plasminogen (PLG), α2-plasmin inhibitor activity (α2pI), plasmin-α2-plasimin inhibitor complex (PPI) and total plasminogen activator inhibitor-1 (t-PAI). All assay was performed by using the reagent manufactured by LSI Medience Corporation. **Results:** The assays using PT, APTT, AT III, FDP and D-dimer showed that the coefficient of variation (C.V.) of within-run repeatability was less than 3.0%, while that of between-day was less than 5.0% in each reagent. The linearity ranges of AT III, FDP and D-dimer were 10% to 170%, 2.5 to 80 μg/mL and 0.3 to 60 μg(D-dimer)/mL, respectively, which were obtained a good recovery rate within ±10% for all sample. Further basic examinations including the other applicable reagents such as TAT are currently under investigation. **Conclusion:** A novel automated coagulation analyzer we developed possess excellent properties for coagulation and fibrinolysis tests, which indicates that this instrument is quite helpful for clinical laboratories.

B-461

Evaluation of Select Assays on the Mindray BA-800M Chemistry Analyzer

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Background: The Mindray™ BA-800M Chemistry Analyzer is a fully automated, discrete, random access chemistry analyzer designed for mid- to large-volume laboratories with a throughput of 800 photometric tests per hour, and up to 1200 tests per hour including ISEs. The analyzer is capable of performing routine and non-routine analysis simultaneously or independently, including chemistry, toxicology, and specialty assays. The sample delivery module has a capacity of 300 samples and the independent sample carousel contains 140 positions for barcoded primary collection tubes or sample cups and offers STAT testing capability. The refrigerated reagent carousel contains 120 reagent positions and can accommodate testing methodologies up to 4 reagents. The reaction carousel consists of a dry bath heating system utilizing glass cuvettes coupled with an 8-step washing/rinsing/drying process. The analyzer offers many features: intuitive software interaction; touch screen monitor; on-board operator's manual with intelligent indexing; intelligent probe management system offering bubble detection, collision protection with auto-recovery, liquid level sensing and clot detection (Sample Probe Only); and remote access diagnostic capability. **Objectives**: The study evaluated the performance of a selection of assays on the Mindray BA-800M Analyzer, using another validated method, the Mindray BS-480 analyzer for most assays, as a reference testing analyzer. The general chemistry reagents are manufactured at the MedTest corporate headquarters located in Canton, Michigan. **Methods**: Analysis was performed based on modified versions of applicable CLSI Protocols. Within Run and Total Precision were determined by running three levels of control material. Within Run Precision was determined by running 20 replicates of controls in a single day. Total Precision was determined by running materials in duplicate across 20 shifts. Accuracy assessment through a correlation of patient samples on the Mindray BA-800M and the comparative analyzers is in process. Limit of Detection for calibrated assays was determined by statistical analysis of response values from five replicates of a low sample and ten replicates of a negative sample. For factored enzymes the limit of detection will be determined by observation of the lowest concentration sample yielding nonzero results. Limit of Quantitation will be determined by assaying a minimum of 40 replicates over at least 5 runs for each sample, using an acceptable precision of CV ≤ 20%. **Results**: Evaluated assays yielded within run precision CVs below 3.9%. Evaluated assays yielded total precision CVs below 7.0%, and most assays had CVs ranging between 0.7% to 5.3%. Limits of detection for evaluated assays were comparable to other similar test systems. Correlation and limit of quantitation studies are currently in process; but preliminary data suggest comparable performance to the Mindray BS-480. **Conclusions**: The performance characteristics of the assays on the Mindray BA-800M Analyzer were comparable to the Mindray BS-480 (or other validated test system). Based upon data generated to date, it can be concluded that the Mindray BA-800M Analyzer is a suitable instrument for use in mid- to large-volume laboratories based upon throughput capabilities and performance.

B-462

UniCel DxI 800 Access Immunoassay System Reliability

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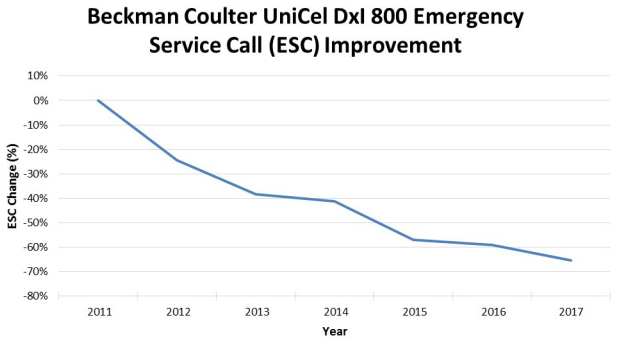
Background: The Beckman Coulter UniCel DxI 800 Access Immunoassay Systems are designed to meet the throughput needs of high volume laboratories. The analyzers offer a broad menu of assays using proven chemiluminescent detection and magnetic particle-separation technology. The reliability of the analyzers is important to customers to minimize downtime. Beckman Coulter has a reliability program in place which drives continuous improvement for better customer experiences. **Methods:** The Beckman Coulter reliability program uses multiple sources for driving improvements. These sources include feedback from both customers and internal personnel including Design, Service, Manufacturing and Supplier Quality. A key input source is customer Emergency Service Calls (ESCs). These are calls which cannot be resolved over the phone and result in dispatching Field Service Engineers to customer sites to resolve issues. ESC information such as service date, analyzer serial number, issue and resolution is recorded in a database which can be used by the reliability team. Reliability engineers analyze all ESCs and group calls into categories based on issue. Categories are investigated to determine root causes of the issues and action plans are developed to address them. Actions are implemented and ESC rates are monitored for the expected impact. **Results:** The reliability program has resulted in a significant decrease in ESCs. The below graph shows the DxI 800 reliability performance from 2011 to 2017. The number of ESCs has decreased every year, with a total decrease of over 65% from 2011 to 2017.



Conclusion: The Beckman Coulter reliability program monitors customer call information, performs data analysis on this information, and then drives improvements into the system and processes. The reliability program has driven a significant improvement in the ESC volume for the DxI 800 from 2011 to 2017. ESC rates during this period have decreased by over 65%. This improved system reliability leads to increased uptime and higher customer satisfaction.

B-463

Specimen Stability and Integrity: Essential factors to quantification of Donor-Derived cfDNA in Transplant recipients

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Background: The release of circulating cell-free DNA (cfDNA) into plasma can serve as an important biomarker for transplant rejection. We developed and validated a next-generation sequencing test (AlloSure®) for quantifying donor-derived cell-free DNA (ddcfDNA). Specimen integrity and stability are essential because total cfDNA is present in low concentrations in the plasma and the dd-cfDNA is a small fraction of the total. Donor and recipient levels must remain stable regardless of storage or handling conditions. Additionally, contamination of the plasma cfDNA by genomic DNA released from the recipient's nucleated blood cells can interfere with the measurement of the dd-cfDNA. For this reason, a hemolysis score based on visual comparision to a hemolysis chart is used as an indicator of potential lysis of nucleated cells. Specimens with more than a trace amount of hemolysis are excluded from testing. The studies reported here evaluated 1) the impact of hemolysis on dd-cfDNA measured in specimens from kidney transplant recipients that expands on studies using simulated hemolysis in plasma from normal healthly volunteers and 2) the stability of cfDNA in plasma stored frozen at -70C. **Methods:** Blood was collected into Streck Cell-Free DNA BCT® collection tubes

from kidney transplant patients and tested using AlloSure. Two tubes were collected from each patient and a hemolysis score was assigned upon receipt of specimens. Extraction was performed using Qiagen's Circulating Nucleic Acid kit. A subset of samples were quantified for total cfDNA using a qPCR method while %dd-cfDNA was calculated using the AlloSure workflow. In the plasma stability study, panels were created from normal healthy volunteers using plasma from one individual spiked into the plasma from another in levels that are consistent with those found in kidney transplant patients. Replicate tubes were stored at -70C for up to 10 months to determine if there were changes to the total amount of cfDNA and donor fraction over time. **Results:** Changes to the level of dd-cfDNA measured were detected when using simulated hemolysis samples with the %dd-cfDNA lower in some hemolyzed samples compared to non-hemolyzed controls. However, our preliminary results using plasma pairs from kidney patients do not show the same effect on the measured dd-cfDNA levels and the %dd-cfDNA levels were similar with differing hemolysis scores. cfDNA in plasma was stable when stored at -70C for up to 10 months after draw. **Conclusion:** Genomic DNA released using simulated hemolysis may not be reflective of the DNA released when hemolysis occurs during routine blood collection and handling. Further understanding of the impact of hemolysis to dd-cfDNA measurement in patient specimens may allow a more relaxed criteria for acceptance of samples showing low levels of hemolysis. The stability of cfDNA in stored plasma allows for use of archived specimens for alternative assessment testing programs and other quality control testing to monitor accuracy and consistency of the test system over time.

B-464

Novel XNA Molecular Clamp Application in NGS Diagnostic Platform OptiSeqTM Cancer Panels

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Background: Next-generation sequencing (NGS) technologies, also known as massively parallel sequencing (MPS), is widely used to detect sequence variations and an array of genetic markers including common and rare variants in cancer genes. NGS and bioinformatics are increasingly used to analyze multiple biomarkers and have been applied in clinical cancer samples. However, one of the challenges in detecting cancer variants with routine NGS analysis is the low frequency in which these mutations are present amongst a background of normal cells. Xenonucleic Acid (XNA) Molecular Clamp Technology is an innovative nucleic acid molecular oligomers that hybridize by Watson-Crick base pairing to target DNA sequences. The xenonucleic acid oligomers are used as molecular clamps during polymerase chain reaction (PCR) to selectively suppress wild-type DNA and amplify mutant DNA. Here, we introduce a highly sensitive NGS Diagnostic Platform Optiseq™ Cancer Panel that combines with the proprietary XNA technology to detect low-frequency somatic variants in cancer samples. **Methods:** Herein we describe a highly sensitive cancer hotspots detection assay to investigate the effects of XNAs on the detected frequency of hotspot variants, particularly variants with low frequency (< 1%), by spiking in a pool of 16 different XNAs in combination or respectively, at varying ratios to the OptiSeq™ Pan-Cancer Panel (DiaCarta) and the QIASeq v3 Human Actionable Solid Tumor Panel (Qiagen). Real patient samples were also tested by spiking in six XNAs individually with XNA Nano Panel (DiaCarta). The assay can be performed directly on patient plasma and FFPE samples and can be readily automated. **Results:** For the OptiSeq™ Pan-Cancer Panel, XNA was found to enrich the cancer hotspot 'driver' and 'drug resistance' mutations (Horizon Discovery) when spiked into a healthy control FFPE DNA. XNA also efficiently enhanced cancer hotspot variants detected by XNA Nano Panel for known positive reference samples. **Conclusion:** XNA technology has the capability to become a powerful NGS diagnostic tool by suppressing abundant wild-type background to detect low-frequency variants of interest in conjunction with targeted cancer panels and allows rapid, efficient and cost-effective determination of the genetic landscape of patient's cancer. Significant progress has been made in characterizing and optimizing the use of XNA in conjunction with oncology NGS and bioinformatics.

B-465

Application of an overall equipment effectiveness indicator in an automated production line for clinical analyses.

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Background: Overall Equipment Effectiveness (OEE) is a measurement of continuous improvement for equipment and productive processes applied in industry to allow for comparison between distinct productive units and the continuous improvement of a manufacturing plant. This methodology is seldom used in the health field, and with the changes that are taking place in clinical analysis laboratories, which are turning into mega-laboratories with automated production lines resembling those in industry, its application will be able to help in the understanding of these transformations, reducing costs and guaranteeing market competitiveness. The present study proposes to apply and make viable the use of the OEE indicator installed in a clinical analysis laboratory.**Methods:**Data collection strategies were designed that would make it possible to faithfully calculate indicators that contribute to the OEE, Availability, Performance and Quality. The following were calculated: total time available, time programmed, time spent producing, time of setup, time spent reloading, mean time stopped, time of production cycle, theoretical production, real production, and good and bad products. For the calculation of the OEE, the contributing factors were considered in multiplied percentage. The data were calculated for two pieces of equipment of the production line, which were the first and last pieces of equipment in the Immunochemical (hormones) line, ADVIA Centaur XP 1 and XP 13, respectively; these, together with the equally automatized sample distribution equipment, are known as Aptio®.**Results:** The results showed Availability of 65.96 % and 64.91 % for the two pieces of equipment analyzed, which means that the laboratory could increase its demand for tests and produce for longer periods, but this also means idle periods and costs for the laboratory. The Performance calculated was 33.64 % (34.4 tests/hour) and 27.55 % (18.1 tests/hour), which is well below the expected value when compared to the manufacturer's description of 100 % performance, which corresponds to 240 tests/hour. In relation to the indicator Quality, the results revealed 96.64 % and 98.02 %, reflecting small margins of error, which means that the quality control and calibrations carried out in both pieces of equipment show a good performance and guarantee a correct final result, which is reflected in the clinical analysis report and the safety of the patient. These multiplied indicators generated an OEE of 21.44 % and 17.53 %, far below the values proposed for industry by Nakajima, who considered the ideal values to be 90.00 % for Availability, 95.00 % for Performance and 99.00 % for Quality, with an OEE of 85.00 %. **Conclusion:**The calculations of the contributing indicators and of the OEE allowed the inefficiencies and strong points present in the laboratory's production line to be analyzed and identified. Just as in industry, the applied OEE was seen to be an exceptional tool for the diagnosis of problems and inefficiencies present in automated laboratory lines. These problems would be difficult to quantify by means of simple strategies, such as the use of internal quality control, which would not detect all the faults that OEE did.

B-466

Evaluation of a New Version Safety Subculture Unit 2

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Background:
Current methods and tools used to subculture blood culture bottles present significant risk for exposure to biohazards and sharps injury. Safety SubCulture Unit 2 (SCU2) is a new subculture device consisting of a needleless cap that fits on blood culture bottles with an internal plastic piercing tip and an external dropper intended for dispensing sample drops from blood culture bottles for subculture and slide preparation.**Methods:** To evaluate ease of use, two (2) studies were performed by experienced laboratory technologists using blood culture bottles positive for bacteria or yeast. Study metrics included:

- device stayed on the bottle,
- initial drop dispensed in ≤10 seconds,
- drop size similar to the original SCU,
- number of drops dispensed

Supplies included forty three (43) SCU2 devices, positive blood cultures in Becton Dickinson and bioMerieux aerobic and anaerobic bottles with and without resins or beads, bioMerieux MP bottle and petri dishes. Positive cultures included categories of Gram Positive and Negative Bacilli (GPB, GNB), Gram Positive Cocci (GPC), Mycobacterium and yeast.
Results:
Data from the two studies were combined and are summarized below;

- device stayed on the bottle: 43 of 43
- initial drop dispensed in ≤10 seconds: 38 of 43

o 5 of 43 produced an initial drop in >10 seconds*, in these 5 cases, bottle type/organism included;

§ 3 aerobic bottles with resins containing GNB

§ 1 aerobic bottle with resins containing GPC

§ 1 aerobic bottle without resins containing GNB

- drop size similar to the original SCU: 42 of 43

• number of drops dispensed: 39 of 43 dispensed 5 or more drops

o 4 dispensed less than 5 drops*, in these 4 cases bottle type/organism included;

§ 4 aerobic bottles with resins containing GNB

Overall performance: SCU2 safely dispensed drops from blood culture bottles *Bottles containing resin and low gas producing organisms (GNB) may increase dispense time and in some cases clog the dispensing tip. Adherence to sampling technique described in the Instructions for Use (IFU) is recommended.
Conclusion:
Safety SubCulture Unit 2 provides an easy to use, efficient method for dispensing samples from blood culture bottles and improves safety by eliminating use of a needle.

B-467

New automated chemiluminescence immunoassays for CSF beta-amyloid determination

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Background: With approved treatments for Alzheimer's disease (AD) on the horizon, diagnostics based on the determination of beta-amyloid 1-42 and beta-amyloid 1-40 in cerebrospinal fluid (CSF) are becoming increasingly important. However, programs like the Alzheimer's Association Quality Control (AAQC) have shown in the past that the field is struggling with high inter-lab variances for these parameters. This makes the comparison of values and determination of global cut points impossible between centers. New, closed test systems could possibly rectify technical issues leading to the observed variances. **Methods:** Magnetic bead-based chemiluminescence immunoassays (CLIA) for beta-amyloid 1-42 and beta-amyloid 1-40 have been developed and validated following the Clinical and Laboratory Standard Institute (CLSI) guidelines on the closed benchtop random access RA Analyzer 15 (Euroimmun). **Results:** The newly developed Euroimmun Beta-Amyloid 1-42 and Beta-Amyloid 1-40 CLIAs with a time-to-first-result protocol of 20 min show inter-assay coefficiants of variation (CV) of 1.5-9.6%. Correlation between the manual Euroimmun Beta-Amyloid 1-42 and Beta-Amyloid 1-40 ELISAs and the new Beta-Amyloid 1-42 and Beta-Amyloid 1-40 CLIAs, respectively, is excellent (99%). **Conclusion:** The newly developed Euroimmun CLIAs for beta-amyloid 1-42 and beta-amyloid 1-40 can be run on a closed random access system (RA Analzyer 15) and show excellent analytical validation data. Significantly reduced hands-on time will further contribute to low variances between labs and a better robustness when including the ratio of beta-amyloid (1-42)/(1-40). However, even though these assays are excellent and accurate diagnostic tools, the field needs to improve and implement global guidelines for pre-analytical sample handling in order to overcome variance that is linked to the sample itself.

B-468

Performance of the Apolipoprotein B assay for use on the Binding Site Optilite® turbidimetric analyser

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The Optilite Apolipoprotein B (Apo B) assay is intended for the quantitative in vitro measurement of Apo B in serum using the Binding Site Optilite analyser to aid in the assessment of cardiovascular disease and lipoprotein metabolism disorders. Apo B is synthesised in the intestine and liver. It is the principal protein component of low density lipoprotein (LDL) and is required for binding LDL particles to the LDL receptor which transports cholesterol to the cells. This may contribute to atherosclerotic plaque build-up in the arteries. There is one Apo B molecule present on each particle of very-low-density lipoprotein, intermediate-density lipoprotein and LDL. Therefore, Apo B concentration provides an effective indication of the total number of potentially atherogenic particles. Apo B determination can be useful in identifying patients who have an increased number of small dense LDL but present with normal LDL cholesterol concentration and could therefore be at increased risk of Coronary Heart Disease. Typically, total cholesterol and triglycerides testing are used for screening coronary risk, but measurement of Apo B, along with other lipoproteins such as lipoprotein (a) and apolipoprotein A-1, can provide further useful information. Here we describe the evaluation of The Binding Site Apolipoprotein B assay for the Optilite analyser. The limit of quantitation (LoQ) for this assay is defined as the bottom of the measuring range, 0.065 g/L. The LoQ verification study was based on CLSI EP17-A. The precision study was based on CLSI EP5-A2. The study was carried out over 5 days using one reagent lot on one analyser. The between run coefficients of variation (CVs) were as follows: 1.27% at 0.69 g/L, 1.43% at 1.25 g/L, 0.89% at 1.67 g/L and 1.93% at 1.99 g/L. A comparison study was performed by analysing 187 samples (including 97 samples with analyte levels within the reference interval) using the Optilite Apolipoprotein B Kit and an alternative commercially available assay. Passing Bablok regression analysis generated the following results: $y = 0.96x + 0.03$ (g/L) (y = Optilite; x = predicate analyser) and correlation coefficient $r = 0.997$ (calculated by linear regression). A linearity study was performed following CLSI EP6-A. The linearity of this assay has been confirmed using a serially diluted serum sample over the range of 0.24 - 3.98 g/L at the standard 1+2 sample dilution with deviation from linearity <10%. An interference study was performed following CLSI EP7-A2. No significant assay interference effects were observed when tested at the standard 1+2 sample dilution with Intralipid (1759 mg/dL), unconjugated bilirubin (61.85 mg/dL), conjugated bilirubin (54.4 mg/dL), haemoglobin (500 mg/dL) or rheumatoid factor (537 IU/ml). No antigen excess was observed up to a level of 3.6 times the top of the calibration curve at the standard 1+2 sample dilution. This is equivalent to 10g/L. In conclusion, the Apolipoprotein B serum assay for the Optilite analyser provides a reliable and precise method for quantifying Apolipoprotein B content in human serum and correlates well with existing methods.

B-469

Human Factor Testing on the Low Level IgG Assay for the Binding Site Optilite® Analyser

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The Binding Site Optilite Low Level IgG Kit is intended for the quantitative in vitro measurement of IgG in urine, cerebrospinal fluid (CSF) and paired CSF and serum samples using the Optilite analyser. Measurement of this immunoglobulin aids in the assessment of the body's lack of ability to resist infectious disease. As part of the validation of this assay, human factor testing was performed using CSF at Labor Berlin, Berlin, Germany and using serum at St Vincent's University Hospital, Dublin, Ireland. Testing was conducted over a 5 day period. Each day a calibration curve was generated and QC samples were assayed twice. Acceptance criteria were provided on the quality control certificate provided with the assay. 3 precision samples were assayed on each day with 5 replicates of each sample in a single run. 5 samples from healthy donors were assayed on each day in singlicate; a different set of 5 samples was used on each day of testing, giving a total of 25 healthy donor samples. 10 samples from patients representing the intended use population of the assay were assayed on each day on the Optilite and results were compared to those generated using the analyser used routinely to measure IgG at each site. 10 different samples were assayed on each day, giving a total of 50 samples from 50 individual patients. All QC results at both sites were within the limits stated on the QC certificate provided with the kit. At Labor Berlin, the 3 precision samples reported mean values of 30.14 mg/L, 77.66 mg/L and 9888.89 mg/L. Total precision CVs were 1.2%, 3.5% and 3.4% respectively. At St Vincent's, the 3 precision samples reported mean values of 33.04 mg/L, 85.88 mg/L and 10729.68 mg/L. Total precision CVs were 3.7%, 2.6% and 4.3% respectively. 22 out of 25 normal samples were within the reference interval when assayed at Labor Berlin, 25 out of 25 samples were within the reference interval when assayed at St Vincent's. The comparison study performed at Labor Berlin using 50 CSF samples gave an ordinary linear regression equation of $1.11x + 2.29$ with a correlation coefficient of $r^2 = 0.987$. The comparison study performed at St Vincent's using 50 serum samples gave an ordinary linear regression equation of $1.09x + 577$ with a correlation coefficient of $r^2 = 0.996$. This testing allowed the laboratories to generate feedback responses based on their experience with the assay in the intended use environment. These responses were returned to Binding Site in the form of a questionnaire. In conclusion, human factor testing of the Low Level IgG assay on the Binding Site Optilite analyser did not identify any issues with the safety or usability of the assay. Both St Vincent's University Hospital and Labor Berlin provided positive feedback on their experiences with the assay, generating positive data that validated the performance of the assay in a clinical laboratory.

B-470

Comparison of Precision Performance across six immunochemistry analyzers

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Objectives: Here we summarize the outcome of a precision study performed at two European sites comparing 13 assays according to CLSI EP05-A3 (5 days) 1x5x5 model. Assays were selected from five indication areas and evaluated on two **cobas e** 801 analytical modules (Roche Diagnostics), ARCHITECT i2000SR (Abbott), UniCel® DxI 800 (Beckman Coulter), ADVIA Centaur XPT / Immulite 2000 XP*i* (Siemens Healthineers) and Liason® XL (DiaSorin). **Methods:** For the precision study, pooled quality control material from Biorad at three different concentration levels per analyte were distributed to both sites. The 13 assays included in this study covered the indication areas Anemia (Ferritin), Bone (PTH), Fertility (Estradiol, Progesterone, Testosterone), Oncology (CEA, CA 125, CA 15-3, CA 19-9, fPSA, tPSA) and Thyroid (FT4, TSH). Testing was done on five days in 5-fold determinations per assay and applied sample pool material. CVs were calculated per site as repeatability and within-lab precision. **Results:** The analyte concentration ranges covered per assay are shown in the table below as mean over all applied methods:

Indication Area	Assay	Unit	Concentration Range	Indication Area	Assay	Unit	Concentration Range
Anemia	Ferritin	µg/L	~ 47.2-641	Bone	PTH	pg/mL	~ 28.8-978
Oncology	CA 125	U/mL	~ 31.4-242	Fertility	Estradiol	pmol/L	~ 368-1762
	CA 15-3	U/mL	~ 21.4-95.1		Progesterone	nmol/L	~ 2.83-76.2
	CA 19-9	U/mL	~ 23.6-219.7		Testosterone	ng/mL	~ 1.93-10.3
	CEA	U/mL	~ 2.57-85.7	Thyroid	FT4	pmol/L	~ 10.7 - 80.6
	fPSA	ng/mL	~ 0.044-10.8		TSH	mIU/L	~ 0.477- 32.1
	tPSA	ng/mL	~ 0.089 - 12.5				

The median repeatability /within-lab CVs calculated over all 13 assays and concentration ranges were: **cobas e** 801 at 1.1% / 1.8%; ARCHITECT i2000SR at 2.9% / 3.0%, UniCel DxI 800 at 3.7% / 4.3%, ADVIA Centaur XPT at 3.1% / 5.0%; Immulite 2000 XP*i* at 4.2% / 5.0%; Liason XL at 2.6% / 3.8%. **Conclusion:** This comprehensive study gives a very good comparison of the precision performance across high throughput immunochemistry analyzers from different manufacturers under standardized conditions.

B-471

Urban inclusion of primary care laboratory services with point of care testing

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Background: City-labs project aims to facilitate in Brussels the access to laboratory tests and allow rapid transmission of results to physicians (general practitioner or specialist) as part of an integrated outpatient care for the chronically ill. The project involves point of care testing and also includes development of digital application for remote monitoring of clinical and biological data. Metrolab Brussels is a transdisciplinary and inter-university laboratory for applied and critical urban research. The Brussels Capital Region through its ERDF program funds both City-labs and Metrolab projects. **Methods:** The team members and researchers of City-labs and Metrolab evaluated different scenarios for an efficient urban inclusion of city-labs structures. Multidisciplinary exchanges were conducted with laboratorians, physicians, urbanists, sociologists and suppliers to define the user's needs and potential features of the solution. **Results:** The metrolab and city-labs researchers identified several key points of attention for the efficient inclusion of city-labs such. The connectivity in public transport and the access by the road will be important, and an easy access by car would be one of the advantages of the City-Lab, including the possibility of parking his vehicle. Our evaluation also allowed to clear two criteria that can guide the choice of location for a City-lab: that of the connectivity of the area (its location at the intersection of axes of different modes of transport) on the one hand, and that of the density or insufficiency of the supply of proximity on the other hand. The ability to drain patients coming from a broad radius requires also attention and an audience with a high mobility potential, moving easily by car or public transport, would be the target audience in priority. Another important point to consider is the insurance of a communication with specialized hospital services to encourage patients concerned about consistency and ongoing monitoring of their medical records. The communication and coordination with general practitioners and ambulatory care services will also represent a priority for the project team members. **Conclusion:** In an era of transition care and interactions between hospital and ambulatory care, the evaluation of the urban inclusion of new care services is mandatory and has to consider mobility and motility as well as communication with healthcare care providers as important determinants.

B-472

Uncertainty of Measurement and Total Analytical Error: Better Together?

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Background: There is an ongoing debate about using uncertainty of measurement versus total analytical error (TAE). Manufacturers aim to address the needs of all customers, some of whom consider measurement uncertainty (MU) and others who use TAE. Both approaches are methods for estimating various forms of error. The approaches could be viewed as complementary, with one being potentially useful to clinicians outside the lab and the other being useful within the testing lab in the context of total allowable error (TEa). An assessment was performed to evaluate the relationship between the two error estimation methods on three clinical chemistry assays - calcium, total cholesterol and glucose - tested on the Abbott Alinity c system. **Methods:** Measurement uncertainty values, TAE and sigma metrics were determined at multiple concentrations across each assay's analytical measuring interval. Total analytical error was estimated using the equation: TAE = 2 × precision + |bias|. The within-laboratory (*i.e.,* intermediate) precision estimates for panels and controls from a 20-day study tested on the Alinity c system were used as the precision values. Bias was estimated from a weighted Deming regression model using data generated from >100 serum samples tested on the Alinity c and ARCHITECT *c*8000 systems. Both studies were conducted at Abbott. Using CLIA TEa goals, sigma values were determined using the equation: sigma = (TEa - |bias|) /precision and plotted on a normalized method decision chart. Measurement uncertainty values were determined using coverage factor (k) of 2, as described in the Guide to Uncertainty of Measurement. Production history and studies performed at Abbott were used to estimate uncertainty associated with repeatability, days, runs, instruments, calibrations, calibrator lots and reagent lots. The relationship between MU and TAE was illustrated in a MU/TAE profile plot showing MU on the Y1-axis, TAE on the Y2-axis and the assay concentration on the X-axis. Additionally, the relationship between MU and sigma metrics was illustrated in a graph showing MU on the Y1-axis, sigma metrics on the Y2-axis and the assay concentration on the X-axis. **Results:** Measurement uncertainty and TAE values were comparable across concentration levels. The performance of >80% (13/16) of the concentration levels evaluated demonstrated >6 sigma performance. One sample for each assay had <6 sigma performance. **Conclusion:** Both methods for characterizing assay quality are valuable. Reporting the measurement uncertainty of a specimen result informs the clinician of the variability around the result, which can be especially important for results near medical decision limits. When assessed versus a TEa goal, total analytical error estimates can be used to categorize the performance of an assay by calculating a sigma metric. This sigma metric can be used in multiple ways, one of which is to help determine appropriate quality control rules for an assay. By reporting measurement uncertainty values with results in addition to using total analytical error and sigma metrics to maintain, monitor and improve quality control, laboratories can take advantage of both methods, thus providing a more comprehensive and higher quality service to patients than either method alone.

B-473

Can we reduce empiric and unnecessary antibiotic use with urinary flow cytometry systems?

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Background: Urinary tract infections (UTIs) are one of the most common infections and rapid diagnosis and treatment are very important. Urine culture is gold standard but time-consuming method to diagnose urinary tract infections. Because the culture results in the earliest 24 hours, physicians start empirical antibiotic treatment. While the empiric treatment can be useful for patients who have UTIs, it causes unnecessary antibiotic use and resistance to bacteria in patients who have no UTIs. In this study, it was aimed to evaluate how much unnecessary antibiotic usage can be avoided by early prediction of negative urine cultures by 'flow cytometry' that is the reference method for cell counting in liquid samples. **Methods:** In this study 1886 urine samples (881 female, 812 male and 143 children) that are accepted in the Central Laboratory were included. At the first stage of study, samples were processed according to standard culture method and then were analysed by UF-1000i (Sysmex, Japan). The cut-off that providing 100% negative predictive values were determined for female, male and child patients. At the second stage, prescription of all patients in the study were investigated with antibiotic prescribing time that were obtained from hospital information system. The antibiotic started in the first 24 hours was accepted as empirically. Unnecessary antibiotic use was accepted in case of antibiotic prescription despite negative or contaminated culture result. **Results:** According to UF-1000i results, 45% of the total samples were not required to culture. All samples that were estimated as negative by UF-1000i were confirmed as negative by culture method. After urine culture request, in first six days, 244 patients were found prescribed antibiotic. Only 17% of 244 patients were observed as positive in urine culture results. When the prescription time and culture reporting time were evaluated together, it was observed that antibiotics were given 44% empirically, 46% unnecessary and 10% relevant to culture results. According to results of UF-1000i urinanalysis, antibiotic prescription could be prevented in 29,6% of patients with empiric therapy and 38,3% of patients with unnecessary antibiotic use. **Conclusion:** As a result, provided that unnecessary antibiotic usage decreases and depending on this cost reduces at least one third rate by usage of UF-1000i that giving rapid results in terms of UTI. We recommend that automated systems like flow cytometric method that provide rapid and accurate diagnosis to patients could be used in together with standart culture methods.

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A global multi-site Sigma-metric assessment of 18 measurands on the Abbott Alinity ci system

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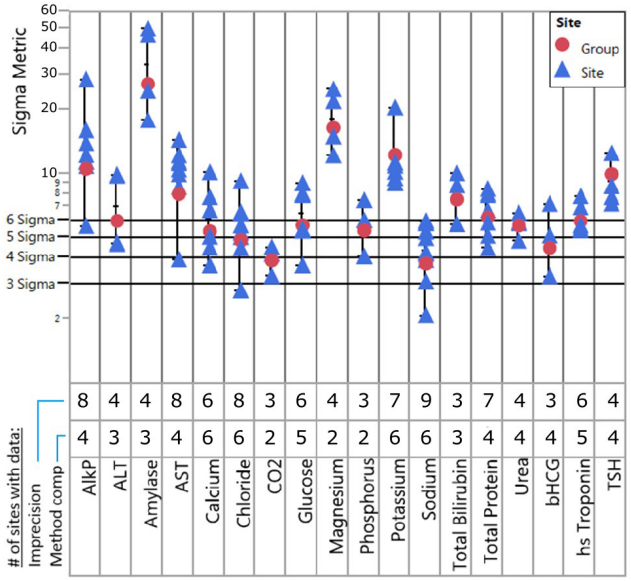
Background: The Abbott Alinity ci system is a next generation, integrated chemistry and immunoassay platform that has been launched in Europe and the Americas, providing an opportunity for evaluation under real-world conditions. The Sigma-metric offers a composite value of assay analytical performance, which was extended to assess performance across multiple sites. **Objective:** To assess multi-site Sigma performance of 3 ISEs, 3 immunoassays and 12 photometric assays on the Alinity ci system across 11 global sites including Austria, Belgium, Canada, France, Germany, Italy, Poland, Spain and UK. **Methods:** Total allowable error (TEa) goals followed the previously defined hierarchy (Westgard et al. Clin Biochem. 2017;50:1216-1221). Precision and method comparison studies were performed based on CLSI guidelines. At the medical decision point for each analyte, bias was calculated from available pooled method comparison data against the ARCHITECT system using Passing-Bablok regression analysis (minimum of two sites). Within-site Sigma-metrics were calculated as (%TEa - |%pooled bias|)/%CV and combined Sigma-metrics were calculated as (%TEa - |%pooled bias|)/%pooled total CV (minimum of 3 sites). **Results:** A total of 97 site-specific and 18 combined Sigma-metrics were calculated. Site-specific Sigma-metrics varied across sites, with 90% of assays performing at least 4 Sigma or higher. Similarly, 16 of 18 combined Sigma-metrics performed greater than 4 Sigma at or near the medical decision point, with Na and CO_2 having a performance just below at 3.7 and 3.9 Sigma, respectively. **Conclusion:** This multi-center study reveals realistic analytical performance of greater than 4 Sigma for majority of assays. Favorable Sigma-metrics were a result of both low imprecision and bias, indicating good comparability between the Alinity and ARCHITECT systems across independent sites but under a single manufacturer. Networked laboratories can use the combined Sigma-metric as a valuable tool to assess between-instrument analytic performance and consistency across multiple sites.



B-475

Multiple mutation detection for risk assessment in patients with breast cancer by using next-generation sequencing

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Background: Breast cancer is recognized as the most common cause of malignancy and cancer death worldwide, the most widely recognized causative genes for breast cancer are BRCA1 and BRCA2, mutations in which lead to hereditary breast cancer and ovarian cancer (HBOC) syndrome, and pose a lifetime risk of cancer between 40% and 80%. However, mutations in the cancer-related BRCA genes are detected in only 2%-3% of the patients with breast cancer. Moreover, risk-assessment studies have identified >90 genes associated with breast cancer incidence, although at least >1000 genes associated with increased risk of breast cancer remain to be identified. Because next-generation sequencing technology allows concurrent sequencing of numerous target genes, diverse cancer-susceptibility genes are now being evaluated, although their significance in clinical practice remains unclear. **Methods:**In this study, we enrolled 60 patients with sporadic breast cancer by using Ion Torrent sequencing technology. The BRCA-plus Panel was designed to target the entire CDS (100% covered) in these 6 key genes: BRCA1, BRCA2, TP53, PIK3CA, ERBB2 (Her2), and PTEN. We focused only on the germline mutations that could help us interpret the genetic phenomena of breast cancer. **Results:** Germline mutations were found to be carried by 9 patients (15%): 3 in BRCA1, 5 in BRCA2, and 1 in TP53. The overall mutation frequency of BRCA1/2 was 13.3%, and the mutation prevalence of non-BRCA genes was 1.6%. By comparison, the tendency of genetic-mutation occurrence in early breast cancer was clearer: before 40 years of age, 26.3% (5/19). The mutation frequency of the Luminal A samples was the highest (21.9%, 7/32), whereas that of the HER2-over-expressing samples was the lowest (0%); mutation frequencies of the Luminal B and triple-negative samples were 8.3% (1/12) and 12.5% (1/8), respectively. **Conclusion:** This study demonstrates the feasibility of using Ion Torrent sequencing technology for reliably detecting gene mutations in clinical practice for guiding individualized drug therapy or combination therapies for breast cancer.

B-476

Performance Evaluation of the Dimension Vista Hemoglobin A1c Assay

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Background: A revised Dimension Vista® Hemoglobin A1c (A1C) assay has been developed* for the measurement of HbA1c in venous whole blood. **Method:** The Dimension Vista A1C assay measures both HbA1c and total hemoglobin. The %HbA1c of the total hemoglobin is calculated and reported. **Total Hemoglobin Measurement:** Whole blood is added to a reaction vessel, which contains a reagent that lyses the red blood cells and converts the released hemoglobin to a derivative with a characteristic absorbance spectrum. An aliquot of the lysed whole blood is then transferred from the reaction vessel to a cuvette, where total hemoglobin concentration is measured photometrically. **Hemoglobin A1c Measurement (turbidimetric inhibition immunoassay):** The same aliquot of the lysed whole blood is used for the measurement of HbA1c. The cuvette contains an anti-HbA1c antibody that reacts with hemoglobin A1c in the sample to form a soluble antigen-antibody complex. A polyhapten reagent is then added, which reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically and is inversely proportional to the concentration of HbA1c. Time to first result is <20 minutes. **Results:** Method comparison of the revised Dimension Vista A1C assay to NGSP yielded the following results: 1.000 (NGSP) using Passing-Bablok regression and 1.010 (NGSP) - 0.068 for Deming regression, n = 126. The assay's performance meets the current requirements for NGSP certification (≤ ±6.0% recovery bias) and would also meet the upcoming NGSP requirement of ≤±5.0% recovery bias. Within-lab reproducibility was ≤2.0% CV at NGSP target values of 5.0, 6.5, 8.0, and 12.0% HbA1c across three kit lots tested on three instruments each for each type of Dimension Vista System. The maximum total error at these same levels was found to be 5.4%. The assay is linear across the assay range of 3.8-14.0% HbA1c. No significant interference bias (less than or equal to ±5.0%) was observed for hemoglobin variants C, D, E, S, or A2. **Conclusion:** The Dimension Vista A1C assay from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

B-477

Performance Evaluation of the Dimension Hemoglobin A1c Assay

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Background: A revised Hemoglobin A1c (A1C) Assay for the Dimension® clinical chemistry system has been developed* for the measurement of HbA1c in venous whole blood. **Method:** The Dimension A1C Assay measures both HbA1c and total hemoglobin. The %HbA1c of the total hemoglobin is calculated and reported. **Total Hemoglobin Measurement**: Whole blood is added to the first cuvette, which contains a reagent that lyses the red blood cells and converts the released hemoglobin to a derivative with a characteristic absorbance spectrum. An aliquot of the lysed whole blood is then transferred to the second cuvette, where total hemoglobin concentration is measured photometrically. **Hemoglobin A1c measurement (turbidimetric inhibition immunoassay):** The same aliquot of the lysed whole blood is used for the measurement of HbA1c. The second cuvette contains an anti-HbA1c antibody that reacts with hemoglobin A1c in the sample to form a soluble antigen-antibody complex. A polyhapten reagent is then added, which reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically and is inversely proportional to the concentration of HbA1c. Time to first result is <10 minutes. **Results:** Method comparison of the revised Dimension A1C Assay to NGSP yielded the following results: 0.991 (NGSP) - 0.034 (Passing-Bablok) and 0.979 (NGSP) + 0.046 (Deming), n = 121. The assay's performance meets the current requirements for NGSP certification (≤ ±6.0% recovery bias) and would also meet the upcoming NGSP requirement of ≤ ±5.0% recovery bias.. Within-lab reproducibility was ≤1.9% CV at target values of 5.0, 6.5, 8.0, and 12.0% HbA1c across three kit lots, each tested on three instruments. Maximum total error at these same levels was found to be 4.1%. The assay is linear across the assay range of 3.8-14.0% HbA1c. No significant interference bias (≤±5.0%) was observed for hemoglobin variants HbC, HbD, HbE, HbS, or HbA2. **Conclusion:** The Hemoglobin A1c (A1C) Assay for the Dimension® clinical chemistry system from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

B-478

EVALUATION OF NON INFERIORITY EFFECTIVENESS OF HYDROGEN PEROXIDE AND SILVER CATIONS DISINFECTION SYSTEM VS ACTIVE CHLORIDE FOR ERADICATION OF MULTIDRUG RESISTANT ORGANISMS

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Background: The objective of this study was to evaluate the non inferiority effectiveness of a disinfection system based on a micro-nebulization of hydrogen peroxide and silver cations vs. active chloride , by monitoring the reduction of microbial contamination of room surfaces. **Methods:** Active chloride (5.000 ppm) and saturated steam vapor (180 C°), vs. decontamination system based on a solution of 5-8%hydrogen peroxide and 60 ppm active silver ions (1mL/m^3 intensity of treatment) were compared. Two beds 26 rooms located in different wards were previously occupied by patients infected by: methicillin-resistant *Staphylococcus aureus* (MRSA) (4 cases); vancomycin-resistant *Enterococcus faecium* (VRE) (2 cases); extensively drug-resistant (XDR) *Acinetobacter baumannii* (2 cases); metallo-beta-lactamase-producing (MBL) *Pseudomonas aeruginosa* (2 cases)*; Kleibsiella* carbapenemase) producing (KPC) (4 cases); extended-spectrum *beta*-lactamase producing (ESBL) *Klebsiella pneumoniae* (2 cases), ESBL-*Escherichia coli* (2 cases), *Stenotrophomonas maltophilia* with phenotype of resistance to trimethoprim-sulfamethoxazole (2 cases) respectively. Environment and medical equipment disinfection procedures were performed prior to a new bed occupancy in addition to routine cleaning activities by the cleaner staff. Surface samples were taken from 10 high touch environmental surfaces which included: room door handle, headboard, footboard, bed frame, bedside table top, bedside table handle, light switch, floor corner, sink taps, soap dispenser. Microbial colonisation was assessed at Time 0 (T0) before cleaning, T1 immediately after cleaning and T2 after disinfection procedures, by use of pre-moistened with sterile saline cotton tipped sterile swabs. The swabs were used to sample surface areas approximately 57 cm^2 by standardized swabbing in two directions at right angles. All swabs were inoculated on blood agar plates and in broth and incubated for 48 hours at 37°C. Cleaning effectiveness of surfaces was evaluated by quantifying the total number of aerobic organisms from a sampled area (total aerobic colony count) and expressed in colony forming units (CFU) per cm^2. Organisms were identified by standard microbiological methods. **Results:** 780 surface samples were collected as follows: 600 from rooms treated with hydrogen peroxide and 180 with active chloride and steam vapor, respectively. Before cleaning the surfaces all samples collected in the rooms resulted colonised, with an average density of mesophile organisms up to 56 CFU/57 cm2 (range 0-400). MDROs were isolated from samples collected in 20/26 rooms respectively. After manual cleaning with detergent followed by active chloride disinfection, an average density of organisms of 15 CFU/57 cm2 (range 0-270) was recorded. MDROs were found from samples collected in 2 rooms but only after an enrichment step. After heated saturated steam vapor disinfection, an average density of organisms of of 15 CFU/57 cm2 (range 1-30) was observed. MRSA were found from samples collected in 2 rooms. After hydrogen peroxide disinfection, a density of bacteria in the range of 0 and 3 CFU/57 cm^2 was observed and no MDROs were found. **Conclusion:** . Our data indicate that the hydrogen peroxide and active silver ions disinfection system, together with the manual cleaning procedures, is non inferior vs. active chloride based procedure. Hydrogen peroxide resulted effective in minimizing the overall microbial load on the hospital room surfaces and in eradicating MDROs

B-479

Evaluation of the Freelite Mx™ Kappa Free and Lambda Free assays on The Binding Site Optilite® turbidimetric analyser using CSF samples

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Inflammation of the central nervous system caused by infections or autoimmune disorders can lead to the synthesis of intrathecal immunoglobulins. Immunoglobulin free light chains (FLCs) are typically secreted along with intact immunoglobulins from plasma cells and if produced intrathecally accumulate locally in CSF. The measurement of FLCs in CSF is a potentially sensitive marker of intrathecal immunoglobulin synthesis and elevated levels of free light chains in CSF may be indicative of central nervous system diseases. Performance characteristics of serum and urine Freelite assays have previously been described. Here we discuss the performance of Free-

lite Mx Kappa Free and Freelite Mx Lambda Free for the detection and quantification of FLCs in CSF on The Binding Site Optilite analyser. A linearity study performed according to CLSI-EP06 guidelines verified that the κ-FLC assay was linear over the measuring range of 0.33 - 12.7 mg/L at the 1+0 analyser dilution recommended for CSF analysis. The λ-FLC assay was also demonstrated to be linear using a serially diluted CSF sample over the measuring range of 0.74 - 17.4 mg/L at the 1+0 sample dilution; with all results being within 10% of expected values. Reference range analysis using 24 oligoclonal banding (OCB) negative CSF samples deemed the κ reference interval to be <0.1 - 1.96 mg/L whereas the reference interval for λ-FLC was not quantifiable. Precision studies were performed based on the CLSI approved guideline EP5-A2, testing two CSF samples on a single kit lot over three analysers and five days. The total precision coefficients of variation (CVs) were 9.4% at 0.51 mg/L and 6.8% at 10.79 mg/L for κ-FLC assessment, and 12.2% at 1.06 mg/L and 5.9% at 15.35 mg/L for λ-FLC assessment. A comparison study was performed by analysing 81 CSF samples using the Optilite Freelite Mx Kappa Free kit and an alternative commercially available assay. Statistical analysis demonstrated good agreement between the assays using Passing and Bablok analysis (y = 0.98x - 0.00) and linear regression (r=0.974). A comparison study was performed by analysing 99 CSF samples using the Optilite Freelite Mx Lambda Free kit and an alternative commercially available assay. Statistical analysis demonstrated good agreement between the assays using Passing and Bablok analysis (y = 0.95x - 0.21) and linear regression (r=0.996). Interference testing was performed according to CLSI EP7-A2 guidelines using a CSF sample close to both Kappa and Lambda medical decision points. No significant assay interference was observed with acetaminophen (1324µmol/L), acetylsalicylic acid (3.62mmol/L), haemoglobin (2.5g/L), conjugated bilirubin (200mg/L) and unconjugated bilirubin (200mg/L). We conclude that the Optilite Freelite Mx Kappa Free and Freelite Mx Lambda Free kits provide a reliable, accurate and precise method for quantifying free light chains in CSF and would be useful in identifying patients with central nervous system diseases.

B-480

Performance of the Lipoprotein (a) assay for use on the Binding Site Optilite® turbidimetric analyser

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The Optilite Lipoprotein (a) [Lp(a)] Reagent is intended for the quantitative in vitro measurement of Lp(a) in serum using the Binding Site Optilite analyser to aid in the assessment of lipid disorders and risk of atherosclerotic cardiovascular disease. Lp(a) is a lipoprotein found in plasma. It consists of a cholesterol-rich low density lipoprotein (LDL) molecule bound to one molecule of apolipoprotein B100 with an additional protein, apolipoprotein (a) [apo(a)], attached via a disulphide bond. Apo(a) can vary in size depending upon the isoform. Apo(a) may inhibit fibrinolysis by competing with plasminogen due to its considerable structural homology; this effect is not observed with LDL free of apo(a). Lp(a) is considered an atherogenic risk factor marker, independent of other lipid parameter markers and exogenous factors such as diet. Increased Lp(a) levels have a high predictive value for coronary heart disease (CHD), especially in combination with elevated LDL cholesterol. Whilst the determination of total cholesterol and triglycerides is used for coronary risk screening, measurement of Lp(a), alongside LDL-cholesterol, HDL-cholesterol, apolipoprotein A-1 and apolipoprotein B, is a valuable tool in differential diagnosis of CHD. Here we describe the quantification and performance of the immunoassay used to detect Lp(a) on the Binding Site Optilite analyser. A linearity study was performed following CLSI Approved Guideline EP6-A. The linearity of this assay has been confirmed at the standard 1+3 using a serially diluted serum sample over the range of 9.63 - 322.73 nmol/L with deviation from linearity <10%. A comparison study was performed by analysing 138 samples (including 103 samples with analyte levels within the reference interval) using the Optilite Lipoprotein (a) assay and an alternative commercially available assay. A Passing and Bablok regression of Y=0.99x + 2.20 was obtained with a correlation coefficient of r=0.999 generated via linear regression. A precision study based on CLSI guideline EP05-A2 was performed over a 5 day period with 2 runs per day. Precision was assessed using 4 samples with different analyte concentrations. The between run coefficients of variation (CVs) were as follows: 1.84% at 39.725 nmol/L; 1.94% at 52.440 nmol/L; 1.85% at 109.195 nmol/L; 1.67% at 155.655 nmol/L. An interference study was performed according to CLSI guideline EP7-A2. Interferents tested include Intralipid (2000 mg/dL), conjugated bilirubin (55.0 mg/dL) unconjugated bilirubin (69.1 mg/dL), haemoglobin (575 mg/dL) and Rheumatoid Factor (520 IU/mL). No significant assay interference was observed when performed at the standard 1+3 sample dilution. In conclusion, the Lipoprotein (a) assay for the Optilite provides a reliable and precise method for quantifying Lp(a) content in human serum and correlates well with existing methods.

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Performance Evaluation of ARCHITECT HSV-1 and HSV-2 IgG Assays on the ARCHITECT Analyzer

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Background: Herpes infection can be caused by both herpes simplex type 1 (HSV-1) and 2 (HSV-2) viruses. HSV-1 infection causes recurrent skin blistering, mainly affecting the facial area, although it can occasionally cause genital herpes. HSV-2 infection is the main cause of genital herpes, a recurrent ulceration in the genital area. When vertically transmitted, HSV-2 may cause neonatal herpes that may result in neurological damage or death. Both, HSV-1 and HSV-2 infections are chronic infections, highly prevalent and widespread throughout the world. HSV diagnosis and subtype differentiation is essential for the disease management. ARCHITECT HSV-1 and HSV-2 IgG assays are chemiluminescent, two-step immunoassays for the qualitative detection of antibodies against HSV-1 and HSV-2 in human serum and plasma. The aim of this study was to evaluate the assays performance on the ARCHITECT Analyzer. **Methods:** Performance was assessed by comparing the new ARCHITECT assays to the BIO-FLASH® HSV-1 and HSV-2 IgG assays, using two subsets of serum samples (549 for HSV-1, and 529 for HSV-2). Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated according to CLSI EP12-A2. For assessment of the assay standardization, sample subsets with samples mainly within the assays critical zone were selected (72 for HSV-1 and 63 for HSV-2) and tested with HerpeSelect® 1 and 2 Immunoblot IgG method. Assuming concordant results between assays in samples beyond the critical zone (0.50-5.00 S/CO in HSV-1, and 0.30-3.00 S/CO in HSV-2), ROC analysis was applied to estimate optimal PPA and NPA against Immunoblot assay. Precision was also evaluated following a 10 days x 2 run x 2 replicates design as per CLSI EP15-A3. **Results:** For ARCHITECT HSV-1 IgG assay, PPA and NPA compared to the BIO-FLASH assay were 99.7% and 98.3% respectively. After further testing of samples within the assays critical zone, PPA and NPA compared to Immunoblot were 97.8% and 91.9%, respectively. Using ROC curves, an optimal cut-off for re-standardization of the ARCHITECT HSV-1 IgG assay was calculated at 1.70 S/CO (PPA and NPA of 96.7% and 95.7% compared to Immunoblot, respectively). Total imprecision for ARCHITECT HSV-1 IgG assay was found to be 0.020 SD for the Negative Control (0.31 S/CO) and 5.5%CV for the Positive Control (2.88 S/CO). For ARCHITECT HSV-2 IgG assay, PPA and NPA compared to the BIO-FLASH assay were 98.1% and 96.6%, respectively. After testing of samples within the critical zone, PPA and NPA compared to Immunoblot were 96.0% and 97.4%, respectively. An optimal cut-off for re-standardization of the ARCHITECT HSV-2 IgG assay was calculated at 0.79 S/CO (PPA and NPA of 98.9% and 96.9% compared to Immunoblot, respectively). Total imprecision for ARCHITECT HSV-2 IgG assay was found to be 0.013 SD for the Negative Control (0.30 S/CO) and 5.0%CV for the Positive Control (3.13 S/CO). **Conclusion:** After re-standardization, the ARCHITECT HSV-1 and HSV-2 IgG assays show suitable performance in terms of agreement to other commercially available methods, which together with the excellent features of the ARCHITECT analyzer, make these assays a remarkable choice for routine use in clinical laboratories.

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Performance characteristics of new UIBC reagent on ARCHITECT cSystems

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OBJECTIVE: To assess the performance characteristics of the new Abbott liquid Unsaturated Iron Binding Capacity (UIBC) assay (LN 04R29) in comparison to the lyophilized Abbott UIBC assay (LN 04P79) on the ARCHITECT *c*System instruments. **RELEVANCE:** The new ready to use liquid UIBC assay (LN 04R29), offered as a replacement for the lyophilized predicate UIBC assay (LN 04P79), is expected to display an improved performance in the form of improved measuring interval, calibration interval and precision profile. **METHODOLOGY:** The UIBC assay (LN 04R29) uses the same methodology as the predicate UIBC assay (LN 04P79), which is Ferene methodology. This methodology comprises of addition of a known concentration of iron to a serum or plasma sample, followed by *3-(2-pyridyl)-5,6-bis-[2-(5-furylsulfonic*

acid)]-1,2,4-triazine (Ferene-S) and a reducing agent. The residual iron from the first step of transferrin saturation forms a stable, reduced, ferrous complex with Ferene-S in the second step. The color intensity of this complex, measurable at 604 nm, is directly proportional to the unbound excess iron-binding capacity. **VALIDATION:** The interference of hemoglobin, intralipid, conjugated bilirubin, unconjugated bilirubin, triglycerides, total protein and rheumatoid factor with UIBC assay was evaluated using a low and a high level of analyte concentration. Analyte recovery within ±10% for analyte concentration greater than or equal to 143µg/dL or within ±14µg/dL for concentration less than 143µg/dL was deemed acceptable. Passing interferent levels for the low level of analyte were found to be 62mg/dL of Hemoglobin, 1000mg/dL of Intralipid, 59mg/dL of conjugated bilirubin, 53mg/dL of unconjugated bilirubin, 901mg/dL of triglycerides, 13.2g/dL of total protein and 100IU/mL of rheumatoid factor respectively. The table below displays the comparison of critical performance characteristics of the new UIBC assay (LN 04R29) and the predicate assay UIBC (LN 04P79).

Comparison of UIBC (LN 04R29) against predicate UIBC (LN 04P79)		
Characteristic	**LN 04R29**	**LN 04P79**
Sample Type	Serum and plasma	Serum and plasma
Limit of Quantitation (µg/dL)	≤ 25	≤ 41
Linearity and Measuring Interval (µg/dL)	25 - 500	41 - 500
Precision	≤ 7% or ≤10µg/dL	≤ 13.3%
Calibration Interval (hours)	168	24
Method Comparison	**LN 04R29 vs. LN 4P79**	
	N	109
	R	0.995
	Equation	Y = 8.03 + 0.9736X
	Range (µg/dL)	31.5 to 458.8

CONCLUSIONS: The new ready to use liquid UIBC assay (LN 04R29) demonstrates better performance in comparison to the predicate method (LN 04P79).

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Performance Evaluation of Atellica IM 1600 Analyzer Assays in a Clinical Chemistry Laboratory

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Background: At our institution, studies were performed to assess the analytical performance of immunoassays (IM) for the Atellica IM Analyzer with respect to verification of precision and linearity, and method comparison with Siemens current assays on ADVIA Centaur® XP System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material ranged up to nine depending on the assay. For each assay, three replicates of each sample level were assayed. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. **Results:** Within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) IM CVs ranged from 1.0% to 7.5% and total (within lab) IM CVs from 0.5% to 7.5%. Linearity studies were performed for all assays. Precision and method comparison studies are summarized below.

		Precision			Method Comparison
Atellica IM Analyzer Assay	**Units**	**Mean concentrations (low, high)**	**Within run %CV(SD) (low, high)**	**Total %CV(SD) (low, high)**	**Atellica IM Analyzer vs. ADVIA Centaur XP System**
Fer	ng/mL	29.43, 304.26	2.6(0.77), 1.0(3.18	2.6(0.77), 1.1(3.37)	*
VitD	ng/mL	16.06 95.86	5.4(0.87) 1.7(1.62)	5.4(0.87) 1.7(1.62)	*
PSA†	ng/mL	0.13, 15.13	4.2(0.01), 2.2(0.34)	4.2(0.01), 2.2(0.34)	0.992x-0.010
eE2	pg/mL	33.84, 948.34	5.8(1.96), 2.6(25.02)	5.8(1.96), 2.6(25.02)	1.102x-10.095
ThCG†	mIU/mL	5.30, 380.48	3.7(0.19), 1.9(7.07)	3.7(0.20), 1.9(7.07)	1.017x+1.734
PRGE	ng/mL	20.51, 5853.80	1.9(0.39), 0.5(27.26)	1.9(0.39), 0.5(27.26)	*
TSTII	ng/dL	135.54, 1132.11	2.2(2.92), 2.7(30.36)	2.3(3.05), 3.5(39.28	*
TSH3-UL	mIU/mL	0.64, 27.29	1.4(0.01, 1.7(0.46)	1.4(0.01), 1.8(0.48)	1.019x-0.015

*Method comparison not done. † Not available for sale in the U.S. Future availability cannot be guaranteed. **Conclusions:** All assays tested on the Atellica IM 1600 Analyzer demonstrated good precision and correlation to the current ADVIA Centaur XP System assays. The precision results were consistent with manufacturer's claims.

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Performance Evaluation of the Atellica CH 930 Analyzer Assays in a Clinical Chemistry Laboratory

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Background: Studies were performed at our institution to assess the analytical performance of clinical chemistry (CH) assays for the Atellica® CH 930 Analyzer with respect to verification of precision and linearity, and method comparison with Siemens current assays on the ADVIA® 1800 System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material (LGC Maine Standards) ranged up to six depending on the assay. For each assay, three replicates of each sample level were assayed. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. **Results:** Within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) CH CVs ranged from 0.2% to 4.8% and total (within lab) CH CVs from 0.4% to 8.2%. Linearity studies were performed for all assays. Precision and method comparison studies are summarized below.

	Precision			Method Comparison		Precision			Method Comparison
Atellica CH 930 Analyzer Assay	**Mean concentrations (low, high)**	**Within run %CV (SD) (low, high)**	**Total %CV (SD) (low, high)**	**Atellica CH 930 Analyzer vs ADVIA 1800 System**	**Atellica CH 930 Analyzer Assay**	**Mean concentrations (low, high)**	**Within run %CV (SD) (low, high)**	**Total %CV (SD) (low, high)**	**Atellica CH 930 Analyzer vs ADVIA 1800 System**
GluH_3	59.04, 348.00 mg/dL	0.5(0.32), 0.3(1.17)	0.6(0.36), 0.5(1.59)	0.979x - 0.45 mg/dL	TP	4.05, 7.15 g/dL	1.1(0.04), 0.7(0.05)	1.3(0.05), 0.8(0.06)	0.958x + 0.022 g/dL
Chol_2	108.20, 279.12 mg/dL	0.7(0.72), 0.7(1.88)	1.0(1.05), 0.8(2.33)	0.935x - 1.488 mg/dL	Alb	2.59, 4.39 g/dL	1.3(0.03), 1.0(0.04)	1.6(0.04), 1.2(0.05)	0.970x + 0.147 g/dL
UN_c	13.88, 72.64 mg/dL	2.3(0.35), 0.7(0.51)	2.3(0.35), 1.3(0.91)	1.005x - 0.988 mg/dL	Trig	93.40, 212.32 mg/dL	0.8(0.75), 0.6(1.30)	1.3(1.18), 0.8(1.77)	1.090x - 6.853 mg/dL
Crea_2	0.78, 6.44 mg/dL	1.1(0.01), 0.4(0.02)	1.1(0.01) 0.7(0.05)	1.045x - 0.007 mg/dL	TBil_2	0.66, 7.61 mg/dL	4.8(0.03), 0.6(0.05)	8.2(0.05), 0.8(0.06)	1.036 + 0.046 mg/dL
Ca	5.51, 13.45 mg/dL	1.9(0.11), 1.1(0.14)	2.5(0.14), 1.2(0.15)	1.011x - 0.090 mg/dL	Na	113.88, 157.48 mEq/L	0.4(0.40), 0.3(0.45)	0.5(0.55), 0.4(0.61)	1.000x - 0.100 mmol/L
AST	42.52, 293.28 U/L	1.8(0.76), 0.4(1.03)	1.8(0.76), 1.1(3.25)	1.050x - 1.729 U/L	K	2.60, 7.33 mEq/L	0.3(0.01), 0.2(0.02)	0.5(0.01), 0.4(0.03)	0.973x - 0.029 mmol/L
ALT	32.20, 214.04 U/L	2.6(0.85), 0.5(1.02)	2.7(0.87), 0.7(1.39)	1.073x + 0.368 U/L	Cl	78.00, 120.72 mEq/L	0.4(0.30), 0.2(0.28)	0.4(0.30, 0.7(0.80)	0.990x + 1.404 mmol/L
D-HDL	29.42, 82.96 mg/dL	1.7(0.51), 0.6(0.46)	1.7(0.51), 0.6(0.53)	1.048x + 0.560 mg/dL	APO A1	93.8, 126.1 mg/dL	0.7(0.69), 1.0(1.25)	2.1(1.96), 1.0,(1.27)	1.035x + 1.746 mg/dL
DLDL	61.43, 160.11 mg/dL	0.6(0.37), 0.6(0.98)	0.7(0.45), 0.7(1.14)	1.067x - 0.284 mg/dL	APO B	39.52, 133.00 mg/dL	1.4(0.57), 0.7(0.94)	2.2(0.86), 1.0(1.31)	0.967x - 8.282 mg/dL

Conclusions: All assays tested on the Atellica CH 930 Analyzer demonstrated good precision and correlation to the current ADVIA 1800 System assays. The precision results were consistent with manufacturer's claims.

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Evaluation of on-board storage and method performance for 8 assays on the Abbott Alinity ci integrated analyzer

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Background The Alinity ci is a next generation integrated system providing a standalone platform for continuous testing of chemistry and immunoassay tests. The Alinity ci also possesses a novel temperature-controlled, on-board storage (OBS) functionality for quality control (QC) and calibrator materials for improved ease-of-use. **Objectives** (1) To evaluate the Alinity ci for precision on the Alinity ci system. (2) To evaluate method comparison against the Abbott ARCHITECT system. (3) To determine the performance of OBS of QC materials compared to conventional off-board storage. **Methods** Eight assays were evaluated using the serum application: 3 ISE (Sodium, Potassium, and Chloride), 3 chemistry (Magnesium, Glucose, Creatinine), and 2 immunoassays (TSH, 25-OH vitamin D). Precision and method comparison studies were performed according to CLSI guidelines. Method comparison was performed in duplicate for both ARCHITECT and Alinity systems over 3 days with a minimum of 100 samples per day. For on-board storage performance, Alinity Technopath Serum S Plus controls for Sodium, Potassium, Chloride, and Glucose, at 3 levels of QC were tested (n=20 per level) for up to 6 days and compared against typical off-board storage condition in a refrigerator. **Results** Precision and method comparison data is summarized in the table below. OBS of QC materials performed similarly to the materials placed under conventional storage. Within-day absolute percent difference was ≤1.6% (Range: -0.4% to 1.6%) for all materials, and absolute percent difference in measured levels from the initial day of off-board storage, difference was ≤1.6% (Range: -0.9% to 1.6%). **Conclusion** The Alinity ci shows excellent real-world performance that is comparable to the ARCHITECT platform with the added storage functionality. On-board QC material storage showed comparable stability and performance to offline storage, thus allowing for improved workflow and ease-of-use.

Summary of Alinity ci Precision and Method Comparison Studies

Analyte	5-day Total Precision						Method Comparison	
	Level 1		Level 2		Level 3		Passing-Bablok Equation	Correlation (R^2)
	Mean	%CV	Mean	%CV	Mean	%CV		
Sodium (mmol/L)	119.0	1.9%	141.7	1.8%	162.0	1.8%	Y=0.999x+1.787	0.989
Potassium (mmol/L)	2.42	1.6%	3.55	1.8%	6.18	2.0%	Y=1.033x-0.042	0.998
Chloride (mmol/L)	77.5	1.8%	92.4	1.8%	105.2	1.8%	Y=1.023x-0.983	0.994
Magnesium (mmol/L)	0.51	2.7%	1.04	0.6%	1.77	0.8%	Y=1.049x-0.002	0.990
Creatinine (mg/dL)	0.66	2.4%	1.95	1.9%	5.74	2.7%	Y=0.987x-0.116	1.000
Glucose (mmol/L)	2.58	2.0%	6.66	1.4%	15.27	0.7%	Y=0.963x+0.008	0.999
TSH (mIU/L)	0.11	1.2%	6.14	1.8%	30.99	2.3%	Y=1.011x+0.001	0.975
25-OH VitD (ng/mL)	19.38	4.0%	40.72	3.0%	78.37	2.8%	Y=0.900x+1.671	0.982

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Cross Reactivity and Interference Results for a Novel 5-plex Panel for the Detection of Acute Kidney Injury

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Background: Acute kidney injury (AKI) is currently classified by serum-based creatinine (e.g., KDIGO) guidelines. However, since creatinine is a lagging index of impending AKI, studies are now underway to qualify a set of biomarkers for detecting drug-induced human kidney injury (DIKI) in clinical trials. Assessment of renal injury using multiple biomarkers is more clinically discriminating than single biomarker analysis. To provide improved turn-around time we have developed a multiplex immunoassay for the urine biomarkers of AKI; KIM-1, NGAL, cystatin C, clusterin and osteopontin (OPN). This work describes the cross reactivity and interference results for our novel multiplex immunoassay. **Methods:** Randox Biochip Array technology was chosen for the development of the five-plex immunoassay using proprietary and commercially available antibodies. Cross reactivity was tested for each analyte from potentially related, but non-panel proteins. For OPN; non-panel biomarkers included enterokinase, thrombin, MMP-7 and MMP-3. For NGAL; α1-acid glycoprotein, α1-microglobulin, HGF, MMP-2, -8 & -9 were tested. For cystatin C; complement C4, MMP-9, cystatin D, HRPG, kininogen, fetuin A & B, cathepsin -L, -B, -D, & -S were tested. For clusterin; Apo-A1, -A2, -B, -B100, -C1, -C2, -D, -E2, -E3, -E4, -H, –M and CLUL-1 were tested. Interfering substances known to be present in urine were titred to determine a non-interfering concentration (within +/- 10%) for cystatin C, clusterin, NGAL and OPN. Interferents included human serum albumin, hemoglobin, bilirubin, pH, glucose, sodium chloride and creatinine. Interference and cross reactivity of non-panel proteins for KIM-1 are pending. **Results:** Cross reactivity was <1% for all tested non-panel, related proteins. Human serum albumin non-interfering concentration was <0.5 mg/mL for clusterin, <3 mg/mL for OPN and <5 mg/mL for cystatin C and NGAL. Hemoglobin non-interfering concentration was <0.5 mg/mL for cystatin C and NGAL, <1 mg/mL for OPN and KIM-1, and <62.5 µg/mL for clusterin. Bilirubin non-interfering concentration was <1 mg/mL for cystatin C and <2 mg/mL for remaining panel biomarkers. Glucose, sodium chloride and creatinine non-interfering concentration was <30 mg/mL, <60 mg/mL and <5 mg/mL, respectively, for all panel biomarkers. **Conclusion:** Interference for substances known to be present in urine are above physiological levels. Cross reactivity results for non-panel proteins indicate no significant cross reactivity. Previously reported data show this multiplex for urine biomarkers is sensitive, precise, linear and has a wide dynamic range. These results and those from the current study demonstrating good selectivity, document the development of an in vitro diagnostic that fills the need for an objective, robust and cost-effective solution to diagnosis and monitoring DIKI and AKI in other settings.

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Universal automated site-specific antibody conjugation

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Background: We previously demonstrated a novel, site-specific, chemoenzymatic, antibody labeling technology that can be applied to essentially any existing antibody with Fc-domain N-linked glycans. We showed this antibody labeling technology has universal application and can be applied to multiple workflows from conjugating antibodies with small organic molecules to large fluorescent Qdot probes and magnetic beads. We have also demonstrated application of the technology to the production of site-specific radioimmune-PET imaging probes, antibody-drug conjugates (ADCs), and tetravalent bi-specific antibodies. The conjugation technology ensures preservation of antigen binding activity, it allows for easy characterization of the sites of labeling, and the reproducibility of labeling is unparalleled when compared to other conventional labeling methods. Three key aspects of the conjugation method, the targeted enzymatic approach, the unprecedented antibody-to-antibody reproducibility of labeling, and the highly-stable labeling reagents, lend the technology to automation. We present here a "load-and-go" automated antibody conjugation platform that yields site-specifically labeled antibodies with high antibody-to-antibody reproducibility. **Methods**: Antibody glycans were modified using a permissive beta-galactosyltransferase enzyme which azide-activates antibody Fc domain glycans using an azide-functionalized sugar substrate. The azide-activated antibodies were conjugated to a fluorescent dibenzocyclooctyne (DIBO) dye in a copperless click reaction. Automated enzymatic azide-activation and DIBO-dye conjugation of the antibodies was performed utilizing magnetic agarose beads. After conjugation, antibodies were eluted from the beads, neutralized, and the degree of labeling (DOL) of the antibodies was determined using fluorescent spectroscopic and fluorescent protein gel scanning methods. **Results**: The entire automation process was completed in a single overnight run. Antibodies were site-specifically labeled with a degree of labeling of 2 or 3 (depending on the sample preparation method used). The antibody-to-antibody reproducibility of labeling was greater than 90% when antibodies were labeled at the same time, during the same run, or between different runs. The preliminary yields, without any optimization, were within the range of 55-65%. **Conclusions**: We present here a "load-and-go" automated antibody labeling platform that site-specifically labels antibodies with high reproducibly while preserving antigen binding activity and antibody integrity. In a single overnight run, up to 5 antibodies can be labeled simultaneously with different payloads. The automated work flow should be compatible with multiple different payloads including, but not limited to, fluorescent dyes, biotin, PET chelators, ADC toxins, and Qdots.

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Development of the Novel and New Multi-Test VITROS® XT Chemistry Products Slides

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Background: A series of new VITROS XT Chemistry Products Slides^ with dual test capability have been developed that are intended to reduce sample size and enhance operational efficiency while maintaining analytical performance versus a conventional single assay test element. The six new XT Slide products are the VITROS XT Chemistry Products UREA-CREA Slides, ALTV-AST Slides, TRIG-CHOL Slides, ALB-TP Slides, GLU-Ca Slides, and TBIL-ALKP Slides. **Methods:** These new slide products are unique in that they allow two tests to be run in a single test element, something that is not currently done in any solution based analytical element in the clinical chemistry laboratory on an automated analyzer. One enhancement of the new Multi-Test Slides is the total sample volume required can be reduced with the smaller test element (0.675 cm^2). The total sample volume to run the twelve XT Slides is 45.6 uL ranging from 2.7 uL for GLU to a maximum of 5.0 uL for ALKP, and decreasing the sample volume by 49% from the previously reduced low sample volume required for these same VITROS Slides (89.5 uL). A second planned improvement is that two tests are run in a single analytical element, therefore increasing the number of tests per hour and increasing the analyzer throughput. For example, the simulated throughput running the comprehensive metabolic panel increases from 681 to 976 tests/hr, a 43% throughput increase. If only the XT Slides were run in the sample mix, a 100% increase in throughput would be realized. **Results:** The new XT Slide products are also planned to maintain the same analytical performance observed with the current VITROS Slide products. We evaluated the accuracy of patient serum samples (UREA: n=124, 2.6 - 106.0 mg/dL; CREA: n=134, 0.13 - 13.68 mg/dL) on the VITROS XT 3400 Chemistry System (in development) compared to the VITROS Chemistry Products CREA Slides. The VITROS XT UREA-CREA Slides showed excellent correlation with the VITROS BUN and CREA Slides. VITROS XT UREA-CREA = 0.999 * VITROS BUN + 0.68; (r) = 0.999 for UREA; VITROS XT UREA-CREA = 0.986 * VITROS CREA - 0.01; (r) = 1.000 for CREA. For the XT ALTV-AST Slide, we evaluated the accuracy of patient serum samples (ALTV: n=132, 5.2 - 744.9 U/L; AST: n=123, 9.5 - 739.4 U/L) on the VITROS XT 3400 Chemistry System (in development) compared to the VITROS Chemistry Products ALTV Slides. The VITROS XT ALTV-AST Slides showed excellent correlation with the VITROS ALTV and AST Slides. VITROS XT ALTV-AST = 0.995 * VITROS ALTV - 0.68; (r) = 0.999 for ALTV; VITROS XT ALTV-AST = 1.001 * VITROS AST + 0.29; (r) = 1.000 for AST. The other four XT Slides show similar accuracy versus their corresponding VITROS Chemistry Products Slides, and all six XT Slides also show precision similar to their corresponding VITROS Chemistry Products Slides. **Conclusion:** With these added features and performance, the new VITROS XT Slides will provide an enhancement to the operational efficiency in the clinical laboratory. ^in development

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Performance Evaluation for an Automated Assay for the Measurement of 17aHydroxyprogesterone on Diasorin's ETI-MAX 3000 Analyzer by ELISA Method

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Background: 17 α - Hydroxyprogesterone (17 OHP) is a steroid produced by the adrenal cortex and gonads. Measurement of 17 OHP is useful indirect indicator of 21-hydroxylase defiency, the most common variety of congenital Adrenal hyperplasia (CAH), as 17 OHP is secreted in abundant excess. Measurement of 17 OHP is therefore valuable in the initial diagnosis of CAH. **Objectives**: The objective of the study is to evaluate the performance of ETI-MAX 3000 using Diagnostics Biochem Canada Inc. (DBC) reagents for both adult and pediatric patients, for the analysis of 17OHP. **Method:** 17OHP method validation was performed using Diagnostics Biochem Canada (DBC) ELISA KIT on ETI-MAX 3000 analyzer from Diasorin. Method validation was done according to the hospital policy which follows CLSI guidelines (EP05-A3/ EP06-A/ EP09-A3/ EP17-A2). Precision study was performed using 40 quality control samples of 2 different concentration for a period of 20 days : Mean , SD and %CV was calculated and compared to the manufacturer recommendation. Sensitivity test was performed using 10 samples of zero 17OHP standard, Mean and +2SD was calculated and compared to analytical sensitivity claimed by manufacturer. Method comparison study was done comparing 20 sample proficiency testing samples to peer group using ELISA method. Slope intercept correlation coefficient was calculated to check the acceptability of the method. Linearity study was done using 7 different concentration standards (calibrator) samples spanning the analytical measurement range (AMR) from 0.11- 20.0 ng/ml. Reference range: 20 normal males and 20 normal females samples were analyzed to verify the manufacturer's recommended reference range. Acceptable criteria is 90% (18 samples must be acceptable out of 20 sample). Age range 21- 48 yrs old for male and range 19- 50 years for female. **Result:** Between days precision study for low QC and high QC % CV was 3.0- 4.7 % respectively. Both %CV were consistent with those claimed by manufacturer. The limit of quantitation was observed at < 0.11 ng/ml which agree with the manufacturer claim. Method comparison acceptable criteria slope 0.90- 1.10, intercept close to zero and r > 0.975. Data was plotted on scatter plot the yield slope was 1.1, Intercept 0.251 and correlation coefficient (r)= 0.998, all results were within acceptable criteria. Linearity: The method was found linear over the AMR of 0.11- 20.0 ng/ml with reportable range up to 160 ng/ml with manual dilution. Reference range study : 100 % of males and females samples result were within the manufacturer's claim for reference range. **Conclusion:**. Overall performance of 17 α – Hydroxyprogesterone on DiaSorin ETI-MAX 3000 was acceptable. It provides reliable results for the required test for both adult and pediatric patients.to help in the diagnosis or monitoring of 17OHP, as the distinguishing characteristics of 21-hydroxylase deficiency is a high serum concentration of 17 α – Hydroxyprogesterone

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Evaluation and comparison of the new Free Testosterone CLIA on the ids/iSYS system

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Background: The measurement of free testosterone (fT) is indicated when imbalance between synthesis of testosterone and its binding proteins is suspected. The goal of our study was to evaluate the analytical performance of a new fT assay. **Methods:** The new test is a competitive, heterogeneous immunoassay based on chemiluminescence detection technology for the quantification of fT in human serum on the ids/iSYS system. Evaluation included intra- and inter-assay precision assessment using control materials and patient sera and method comparison with a Beckman radioimmunoassay. The fT assay was additionally validated using 70 samples of hypogonadal obese men and 30 specimens collected from young athletes. **Results:** The observed imprecision (CV) ranged from 2.6% to 6.6% on replicates of quality control samples provided with the test kit (inter-assay) and from 2.7% to 6.5% on replicates of patient samples distributed over the measurement range (intra-assay). Method comparison resulted in a correlation coefficient (Pearson) of r^2 = 0.9689, slope (Passing-Bablok) of 0.76 (95% CI, 073 - 0.80) and intercept (Passing-Bablok) of 0.15 (95% CI, -0.28 - 0.60). The median of the relative bias observed amounted to -23.5%. The mean fT value in the hypogonadal obese group (28.2 pmol/L, CI95% 26.2 - 30.2) was significantly lower ($p << 0.05$) than in the athletes group (35.6 pmol/L, CI95%, 32.1 -39.1). Comparison between measured and estimated fT shows a median bias of -86.7% which is in accordance with the literature. **Conclusion:** Precision results have been fully satisfying and reflected the manufacturer's declared performance. The observed bias to the current method is stable over the whole measurement range and might be explained by the use of different assay antibodies and reference material. The newly provided reference ranges take this bias into account. The fT assay shows satisfying analytical performance and could be helpful as a complementary biomarker for the diagnostic work-up of hypogonadism in combination with total testosterone and clinical assessment.

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Development of Assays for Micro blood samples: CBC, Biomarker and hormonal assays

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Objective
To modify and perform various assays, Complete blood count (CBC) and Biomarkers/hormonal assays in 100 ul of whole blood, collected using commercial blood collection device.
Relevance
The process of blood collection is painful and involves collection of multiple tubes by venipuncture . This has difficulty in collecting blood in children and elderly patients. Finger pricking has been used to collect whole blood or blood spot in filter paper. This has challenges with red cells and tissue fluid contamination and development of normal reference ranges in capillary blood. Recently, several commercial companies have developed painless blood collection devices. We have investigated one of the devices in blood collection and to perform various routine assays.
Methods
The TAP blood collection device from Seventh sense Biosystems (Medford, MA) is applied to the upper arm region of the patient. It is virtually a painless process utilizing "microneedles" to puncture the skin and gentle vacuum for suction of blood into a chamber containing anticoagulants. The device will only provide 100 ul of sample. Because of the limited sample volume, assays are scaled down or dilution paradigms were utilized to maximize the utility of the sample. The study was done under IRB protocol. The CBC was determined using a Sysmex XS-1000i, Hemoglobin A1C using Beckman AU480 . The remainder of the whole blood was centrifuged and the plasma was used for LC/MS/MS based assays for Vitamin D & testosterone and immunoassays for TSH, PSA and sex hormone binding Globulins (SHBG). Quality control samples were treated similar to the blood samples. The micro samples tested were correlated with conventional venipuncture blood samples.
Results
Whole blood (100 ul) collected using the TAP device, was first used for CBC determination and a small volume is diluted and cells lysed for HbA1c analysis. The rest of the whole blood is centrifuged and an aliquot of plasma was diverted to LC/MS/MS assays for Vitamin D and Testosterone. The remaining plasma was diluted 1:10 in an immunoassay calibrator diluent and was tested for TSH, PSA and SHBG using sensitive manual immunoassays. All assays were validated and analytically correlated with a predicate method using previously tested proficiency samples and human specimens. The manual immunoassay compared against an automated immunoassay analyzer with a correlation of 0.92. The micro volume LC/MS/MS based methods were compared against comparative LC/MS/MS methods that required 10x the amount of sample. The CBC instrument using capillary mode resulted in micro volume requirement. Modified methodologies demonstrated good statistical correlation, good reproducibility (< 15 % CV) and similar reference ranges between the newly developed assays and the predicate methodologies.
Conclusion
Micro sampling of whole blood (100 ul / device) could be achieved using a painless blood collection device. To accommodate small blood sample volume, automated instruments, immunoassays and mass spectrometry assays were modified .These modifications could be used to quantitate various markers used routinely.

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A novel ELISA for the quantification of tau phosphorylated at threonine 181 in cerebrospinal fluid

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Background: The prevalence of dementia constantly increases as the Western populations grow older. Biomarkers in cerebrospinal fluid (CSF) are a valuable diagnostic aid for the diagnosis of Alzheimer's disease (AD). We developed a novel phospho-tau (pTau(181) ELISA for use in routine diagnostic laboratories with good analytical performance, robustness, user-friendliness and the possibility of automation. **Methods:** A 4-hour protocol was developed for the Euroimmun pTau(181) ELISA. The protocol as well as the test kit components were adapted to allow parallel analysis of the complete CSF biomarker profile for AD: Euroimmun ELISAs for the determination of beta-Amyloid (1-42) and (1-40), total Tau and pTau(181). Clinically characterised samples from the University of Magdeburg, Germany, were used for comparison with a reference test, the INNOTEST Phospho-Tau (181P) (Fujirebio). **Results:** Evaluation of the Euroimmun pTau(181) ELISA revealed the following test characteristics: intra-assay coefficient of variation (CV) ranges from 1.3 % to 5.0 %, inter-assay CV from 3.1 % to 4.9 %, whole blood interference tolerated up to 1 % v/v, no hook effect observed up to 100 ng/ml analyte. Manual and automated (Euroimmun Analyzer I) protocols gave similar results. The assay is very specific for the phosphorylation site 181 and showed no cross reactivity against other sites (e. g. 175). INNOTEST Phospho-Tau (181P) and the new Euroimmun pTau(181) ELISA were compared using 110 clinically characterised samples (61 AD patients, 49 disease/healthy controls). The Euroimmun pTau(181) ELISA showed a sensitivity of 93.4 % and a specificity of 83.7 %, while the INNOTEST Phospho-Tau (181P) assay showed a sensitivity of 67.2 % and a specificity of 91.8 % using the same cut-off (61 pg/ml). According to ROC analysis, at a predefined specificty of 91.8 %, the sensitivity of the Euroimmun assay (86.9 %) even outperformed that of the INNOTEST (67.2 %) significantly. **Conclusion:** The novel pTau(181) ELISA was validated for manual as well as automated processing on open ELISA systems such as the Euroimmun Analyzer I. Analytical performance evaluation included intra-assay, inter-assay and interference studies. The assay meets all requirements of a routine diagnostic test and represents an even better alternative to the established INNOTEST ELISA. The 4-hour protocol allows the test to be run in parallel to tests for other Euroimmun neurodegenerative biomarkers, i. e. beta-Amyloid (1-42) and (1-40) as well as total Tau, and enables a shorter time to result compared to ELISAs from other manufacturers. The possibility of automated parallel processing of all classical CSF biomarkers for AD on an open ELISA system is a step towards more reliable and comparable results.

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Application of a Biochip Array to Simultaneously Measure Analytes Related to Metabolic Syndrome in Serum with the Use of the New Random Access, Fully Automated Evidence Evolution Analyser

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Background: Metabolic syndrome, a combination of several metabolic related disorders, is a precursor to many diseases such as cardiovascular disease, type

2 diabetes, stroke, and cancer. The simultaneous detection of analytes related to metabolic syndrome is beneficial in clinical settings. Biochip array technology enables the detection of multiple analytes from a single sample, which increases result output. The aim of this study was to evaluate the applicability of biochip array technology to the simultaneous quantitative measurement of analytes related to metabolic syndrome ferritin, insulin, leptin, Plasminogen Activator Inhibitor-1 (PAI-1) and resistin in serum through the use of a biochip array applied to the fully automated, random access with STAT capabilities Evidence Evolution analyser. This application will facilitate research into metabolic related disorders **Methods:** Simultaneous chemiluminescent sandwich immunoassays, defining discrete test regions on a biochip surface and applied to Evidence Evolution analyser, were employed. Analytical sensitivity, inter-assay precision and serum patient sample (n = 81 for ferritin, n = 90 for insulin and n = 55 for leptin) method comparison studies were conducted. **Results:** Analytical sensitivity values of 2.53 ng/mL for ferritin (assay range 2.53 - 941.74 ng/mL), 0.09 µIU/mL for insulin (assay range 0.09 - 282.18 µIU/mL), 0.68 ng/mL for leptin (assay range 0.68 - 105.02 ng/mL), 0.60 ng/mL for PAI-1 (assay range 0.6 - 152.27 ng/mL) and 0.20 ng/mL for resistin (assay range 0.20 - 68.6 ng/mL). Inter-assay precision for low, medium and high levels of precision material, expressed as CV (%) (n=20) was as follows: 4.0%, 5.6% and 5.9% for ferritin, 10.1%, 8.2% and 9.9% for insulin, 4.0%, 4.8% and 7.3% for leptin, 9.4%, 9.9% and 9.8% for PAI-1and 13.3%, 12.7% and 10.5% for resistin. Serum patient samples were assessed with the biochip array and another commercially available system and the correlation coefficients were r= 0.95 for ferritin, r=0.95 for insulin and r=0.96 for leptin. **Conclusion:** Data indicate that the developed biochip array for application to the new fully automated Evidence Evolution analyser detects simultaneously analytes related to metabolic syndrome (ferritin, insulin, leptin, PAI-1 and resistin) from a single serum sample. This platform presents optimal analytical performance, compares favourably with another system and represents a reliable new analytical tool in the research of metabolic related disorders.

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Automated measurement of plasma free hemoglobin using hemolysis index check function

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Background: Plasma free hemoglobin is an important indicator of intravascular hemolysis. Most clinical laboratories measure plasma free hemoglobin spectrophotometrically using two or three wavelengths. A point-of-care testing device is also available to measure plasma free hemoglobin. The Roche Diagnostics Cobas chemistry analyzers have a function to check hemolysis index (HI) and equations have been developed to estimate plasma free hemoglobin concentrations based on HI. Since the Roche Diagnostics chemistry analyzers' HI check function can directly report hemoglobin concentrations, we aim to determine if the hemoglobin concentrations reported by the hemolysis check function can be used as a measurement of plasma free hemoglobin. **Methods:** The Roche Cobas chemistry analyzers took an aliquot of the lithium heparin plasma and dilute it in saline (0.9 % sodium chloride) to measure the absorbances for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength) and hemoglobin concentrations were calculated. Two samples with low and high concentrations of free hemoglobin were measured 20 times to evaluate within-run and between-run imprecision. Two samples with hemoglobin concentrations of 6 and 11 mg/dL were repeated 20 times to determine the lower limit of quantification. Six samples with known concentrations between 5 and 506 mg/dL were measured induplicate to evaluate the analytical measurement range. Fifty two samples were analyzed with the present method and an existing reference spectrophotometric method to evaluate the correlation between them. Two samples with low and high free hemoglobin concentrations were measured repeatedly in various combinations to evaluate carryover. Bilirubin was added to samples with known free hemoglobin concentrations to evaluate the interference. To evaluate the interference from triglyceride, free hemoglobin concentrations in samples with different lipemic index were determined before and after removing triglyceride by high-speed centrifugation (21,380 g for 15 minutes). **Results:** Within-run and between-run CVs were 2.8-10.1% and 2.1-7.0%, respectively (n = 20). The lower limit of quantification was 11 mg/dL (CV = 8.1%) with the upper limit of analytical measurement range of 506 mg/dL. The results of the present method correlated well with the existing reference spectrophotometric assay: Y (present method) = 1.079X (reference method) - 3.9, r = 0.9996, n = 50). No significant carryover was observed. Bilirubin with a concentration up to 75 mg/dL and lipemic index up to 200 did not show significant interference. Since the present method and the existing method show an excellent correlation, the reference interval for the reference method (0-22 mg/dL) was transferred to the present method. **Conclusion:** The performance of the plasma free hemoglobin measurement directly by the hemolysis index check function on the Roche Cobas chemistry analyzers meets the analytical requirements of the clinical plasma free hemoglobin assays. It is simple, automated, convenient, and cost-effective.

B-495

Performance of the Apolipoprotein A-1 assay for use on the Binding Site Optilite® turbidimetric analyser

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The Optilite Apolipoprotein A-1 (Apo A-1) assay is intended for the quantitative in vitro measurement of Apo A-1 in serum using the Binding Site Optilite analyser to aid in the assessment of lipid disorders and risk of atherosclerotic cardiovascular disease. Apo A-1 is the principal protein component of high density lipoprotein (HDL). Expression of Apo A-1 may be largely responsible for determining the plasma level of HDL. Apo A-1 also functions as a cofactor for lecithin cholesterol acyltransferase, which is vital in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport to the liver. Therefore Apo A-1 and HDL cholesterol (HDL-C) concentrations are thought to be inversely related to risk of coronary heart disease (CHD). Apo A-1 has been shown to be a strong predictor for CHD risk. Typically, total cholesterol and triglycerides testing are used for screening coronary risk, but measurement of Apo A-1, along with other lipoproteins such as lipoprotein (a) and apolipoprotein B, can provide further useful information. Here we describe the evaluation of an Apolipoprotein A-1 serum and plasma assay (manufactured by The Binding Site Ltd, UK) for the Binding Site Optilite analyser. The measuring range of the assay is 0.193 - 2.750g/L at the standard 1+3 dilution, with an overall sensitivity of 0.048g/L at the reflex low 1+0 dilution. A precision study was performed according to CLSI approved guideline EP05-A2 over a period of 5 days using one reagent lot on one analyser. The study was carried out using 4 samples with different analyte concentrations. The between run precision coefficients of variation (CVs) were as follows: 0.81% at 0.42g/L, 1.09% at 1.24g/L, 2.23% at 1.53g/L and 2.37% at 2.33g/L. The acceptance criteria was <4% CV for between run precision. A comparison study to the Hitachi 917 assay was performed using 150 samples ranging from 0.15g/L to 3.51 g/L (Passing and Bablok analysis slope y = 0.94x + 0.02). A Limit of Quantitation (LoQ) verification study was based on CLSI EP17-A. The LoQ for this assay is defined as the bottom of the overall measuring range, 0.048 g/L. A linearity study was performed following CLSI Approved Guideline EP06-A. The assay gave a linear response over the analyte range of 0.09 - 3.74g/L at the standard 1+3 analyser dilution using a serially diluted serum sample. Interference testing was performed following CLSI guideline EP07-A2 using 6 potential drug and metabolite interferents including Intralipid, triglyceride and haemoglobin at 2 testing levels; the medical decision point (1+3 analyser dilution) and a pathological level (1+0 analyser dilution). No significant assay interference effects were observed (all results <10% from a corresponding blank sample). In conclusion, the Apolipoprotein A-1 assay for the Optilite provides a reliable and precise method for quantifying Apo A-1 content in human serum and correlates well with existing methods.

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Performance of the Complement C2 assay for use on the Binding Site SPAPLUS® turbidimetric analyser

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The Human Complement C2 Kit for use on SPAPLUS is intended for the quantitative *in vitro* measurement of human Complement C2 in EDTA plasma and serum using the SPAPLUS analyser. This test should be used in conjunction with other laboratory and clinical findings. C2 is a β1-glycoprotein which forms part of the classical complement pathway. It is cleaved by activated C1s into two fragments, C2a and C2b. The larger fragment of C2 then combines with C4b to produce C3 or C5 convertase. Reduced C2 plasma or serum concentrations may result from either a C2 deficiency or a complement-consumptive process. C2 deficiency is the most common inherited complement component deficiency, and is associated with systemic lupus erythematosis, glomerulonephritis, vasculitis and severe pyogenic infections. Here we describe the performance of an immunoassay for the detection and quantification of Human Complement C2 Kit for use on SPAPLUS. A linearity study was performed following CLSI Approved Guideline EP6-A. The assay was confirmed to be linear over the standard 1/10 measuring range of 4.0 - 45.0 mg/L using a serially diluted EDTA plasma sample. An interference study was performed according to CLSI guideline EP7-A2.

Interferents tested included bilirubin (200 mg/L), haemoglobin (5 g/L), intralipid (500 mg/dL) and triglyceride (1000 mg/dL). No significant assay interference was observed when performed at the standard 1/10 sample dilution. A limit of quantitation (LoQ) study based on CLSI EP17 confirmed a limit of 4.0 mg/L with the total error being <1.0 mg/L. A precision study based on CLSI guideline EP05-A2 was performed over a 21 day period with two runs per day, three reagent lots and three analysers. Precision was assessed using 5 EDTA plasma samples with different analyte concentrations. The total prevision coefficients of variation (CVs) were as follows: 10.9% at 7.509 mg/L, 8.0% at 9.268 mg/L, 6.7% at 11.226 mg/L, 5.5% at 23.631 mg/L and 7.8% at 34.364 mg/L. A comparison study was performed by analysing 119 paired EDTA plasma and serum samples using the SPAPLUS Complement C2 assay and the SPAPLUS CH50 assay. The two assays were observed to have a high degree of relative agreement (98.3%). In conclusion, the Complement C2 assay for the SPAPLUS provides a reliable and precise method for quantifying Complement C2 content in human EDTA plasma and serum. It correlates well with existing complement assays.

B-497

Association between the clusterin level in peripheral blood and its gene polymorphism and Alzheimer disease

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Background: To assess the association between the clusterin level and its gene polymorphism and Alzheimer disease among ethnic Han Chinese in southwest of China **Methods:** A total of 108 patients with AD and 115 healthy controls were enrolled in this study. SNaPshot SNP typing was used to genotype 11 SNP were selected. Serum levels of clusterin were detected by ELISA **Results:** The clustrin level of peripheral blood in AD group was significantly higher than Control group (P = 0.001). The rs3087554 locus of CLU gene was significantly different in the dominant model (P = 0.037, OR = 0.523, *95% CI* = 0.284-0.962), and the locus was significantly difference in the **APOE**ε4-carrying case group and control group (P = 0.036). The other eight loci genotypes and alleles in the case group and control group distribution was no difference. No significant association were found between genotypes and serum clusterin levels. **Conclusion:** The plasma levels of clusterin in AD patients are significantly higher than those in normal controls. This conclusion is consistent with the other reports that clusterin may play a role in the occurrence and development of AD. CC or CT genotype of rs3087554 in CLU gene may be a protective factor of AD and can be a biomarker. No associate was found between the other eight SNPs and AD disease.

B-498

Novel Predictive Biomarker for Monitoring Adverse Reactions to Radiation Therapy

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Background: Radiation treatment is required by 70% of cancer patients, however, there is currently no clinical method for determining the therapeutic response or radiation-induced toxicity that can be used during a course of radiation therapy to personalize the dose for individual patients. The only standard method is CT/PET and/or MRI. This is a major clinical concern for radiation oncologists with so many new agents being approved in combination with radiation therapy. **Methods:** Herein we describe a highly sensitive clinically validated assay that measures the extent of normal tissue damage induced by radiation by quantitation of circulating free DNA (cfDNA) derived from cellular apoptosis detected in plasma of patients undergoing radiation therapy. The assay employs DNA capture probes and SuperbDNA™ signal amplification technology with alkaline phosphatase labeled signaling probes coupled with dioxetane phosphate chemiluminescence detection. The assay can be performed directly on patient plasma samples and can be readily automated. **Results:** The lower limit of detection (LOD) for this assay was shown as 0.39 ng/ml, which equals 7.8 pg human genomic DNA given that 20 ul of samples were loaded. The assay has a wide range of linearity from 0.39 to 50 ng/ml that allows for quantitative measurements of circulating DNA at concentrations expected in cancer patients and healthy individuals. The inter- and intra-assay coefficients of variance were <21.4% and <12%, respectively. Freeze-thaw stability testing showed that the reagents of the assay were stable up to 9 freeze-thaw cycles. Plasma samples from 47 patients with prostate cancer were tested and the levels of circulating DNA in plasma pre-radiotherapy was 11.6 ~ 130.6 ng/ml with a median value of 32.2 ng/ml, significantly higher than the normal plasma which was 5.01 ng/ml. Within 5 days post-radiotherapy, 13 of 47 patients (27.6%) showed >2.0-fold peak increase of circulating DNA levels. In addition, the ratios of post-radiotherapy peak levels to pre- levels in patients receiving X-ray treatment were significantly higher than patients receiving proton treatment, suggesting X-ray caused more toxicity than proton. **Conclusion:** RadTox can be used both for research and clinical testing of plasma samples for patients undergoing radiation therapy for optimization and personalization of treatment.

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Evaluation of a Standard Material Traceable Enzymatic Method Assay for Glycated Albumin: Analytical Performance and Establishment of Reference Values

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Backgound
Glycated Albumin (GA) is an intermediate-term marker useful for the monitoring of glycemic control (preceding 2-3 weeks) in diabetes mellitus patients. We developed the Lucica® Glycated Albumin-L assay (GA assay), a diagnostic reagent for the quantitative measurement of GA that is traceable to standard reference materials based on an enzymatic method. This study is designed to evaluate the performance of the GA assay and develop the reference range of GA in healthy subjects without diabetes.
Methods
The performance studies (Precision/Reproducibility, Linearity, Stability, Interference, Detection limit, and Reference range) were conducted in accordance with *CLSI* Guidelines. The traceability was studied using the Secondary Calibrators (Glycated Albumin Certified Material, JCCRM 611-1, M, H, HH: ReCCS, Japan). For the reference range study, a single-visit 2-sites study was designed. Subjects with HbA1c < 5.7%, and a fasting glucose < 100 mg/dL, and 2-h plasma glucose in 75g OGTT < 140 mg/dL were enrolled in the study as healthy subjects without diabetes. The reference range was constructed based on 2.5 and 97.5 percentiles for the GA data of healthy subjects.
Results
In the single-site precision/reproducibility study, five serum pools were tested two runs per day in duplicates for twenty days. The overall repeatability (%CV) and the overall within-laboratory precision (%CV) were not more than 3.7% and 4.2%, respectively. In the multi-site precision study, three serum pools were tested five replicates per run, one run per day, for five testing days at three different laboratories. The overall reproducibility (%CV) values among the laboratories were not more than 2.5%. The GA value showed good linearity from 173 - 979 mmol/mol across the assay range. The Calibrator and Control were traceable to the secondary calibrator. The shelf-life for the reagents was 12 months when refrigerated within a temperature range between 2 and 8 °C. The open reagent was stable for 1 month. The LoB was 6.9 µmol/L for GA concentration and 3.8 µmol/L for Albumin concentration. The LoD was 7.9 µmol/L for GA concentration and 7.0 µmol/L for Albumin concentration. The LoQ was 9.7 µmol/L for GA concentration and 21.8 µmol/L for ALB concentration. The following substances were found not to interfere at the concentrations indicated (bias < 10%): unconjugated bilirubin up to 20.0 mg/dL, conjugated bilirubin up to 20.0 mg/dL, glucose up to 1000 mg/dL, ascorbic acid up to 100 mg/dL, hemoglobin up to 288 mg/dL, triglycerides up to 1516 mg/dL, and uricacid up to 23.5 mg/dL. The reference range in 262 healthy subjects without diabetes ranged between 183 and 259 mmol/mol.
Conclusion
In conclusion, the results demonstrated that the Lucica Glycated albumin-L assay shows excellent performance and may be a useful diagnostic test for the intermediate-term monitoring of glycemic control in patients with diabetes.

B-500

Accurate and high-throughput,targeted quantification of CpG methylation without DNA extraction and bisulfite treatment

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Background: DNA methylations at specific CpG loci have been increasingly utilized as biomarkers for cancer diagnostics. Targeted quantification of CpG methylation levels currently requires tedious DNA extraction and complex multi-step procedures that prevented clinical applications involving a large number of specimens. In addition, accurate and reproducible quantification of CpG methylation is often difficult using bisulfite-based quantitative assays due to inconsistent C to U conversion and template DNA degradation. Methylation-sensitive endonuclease-based assays such as MS-MLPA bypassed problems with the bisulfite, but still requires DNA extraction, and the presence of unbound probes in reactions and the change of reaction tubes for different enzymatic steps reduces the assay specificity and sensitivity. Here we described a highly reproducible, endonuclease-based,

quantitative and high throughput CpG methylation assay that does not involve DNA extraction and bisulfite conversion, with an ELISA-like workflow using one 96-well plate, and with a multiplex capability of up to 20-30 CpG loci in each well. **Methods:** Blood or FFPE sample are lysed to release target DNAs, which are captured to the bottom of the 96-well plate via sandwich hybridization with multiple contiguous target-specific probes having defined 5'- or 3'-end tail sequences. After removal of unbound probes and the enzymatic ligation of the bound probes, the ligation products spanning each target CpG site are treated either with or without a methylation-sensitive restriction endonuclease, which will cleave at specific unmethylated-cytosine residues while leaving the methylated ones intact. After buffer change, PCR amplification is performed in the same well with a universal primer pair targeting the tail sequences. Quantification of the CpG methylation percentage levels is obtained by comparing between restriction-treated and untreated groups the amount of each amplified products. For single CpG measurement this is achieved via delta Cq with real-time PCR; and for multiple CpG determination this can be achieved via standard PCR and multiplexed quantitative single-base primer extension analysis of the amplified products with MOLDI-TOF mass spectrometry (MassARRAY platform). **Results:** We prepared a test series of SssI-methylated DNA samples with CpG methylation levels ranging from 0%-100%, and measured the degree of methylation in triplicate using our assay with the enzyme HpaII. A linear regression analysis revealed quantitative and reproducible recovery across the entire methylation range, with the root mean square deviation of less than 5.2%, and a slope of 1.016 with an R^2 of 0.993. With a significantly simplified procedure and a much higher throughput, our assay efficiently offered overall better accuracy and consistency of the measured methylation percentage values than obtained with the bisulfite-based, gold standard pyrosequencing of the same samples. The robustness and sensitivity of our assay and its application in quantitative CpG methylation assessment in cancer (both cell lines and clinical samples) as well as in human aging will be presented. **Conclusion:** With no need for nucleic acid purification and bisulfate conversion, our method provides a highly reproducible multiplex quantitative CpG methylation assay suitable for high-throughput clinical applications.

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