SCIENTIFIC POSTER SESSION SCHEDULE

Posters of the accepted abstracts can be viewed in Hall A of McCormick Place, on Tuesday, December 15 and Wednesday, December 16. 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, DECEMBER 15, POSTER SESSIONS

9:30am – 5:00pm

WEDNESDAY, DECEMBER 16, POSTER SESSIONS

9:30am – 5:00pm

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Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Advanced Data Analysis

A-004

Improvements of an Autoverification System for Clinical Chemistry Test Results using Big Data Strategy and Multicenter Validation Process

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Background: The autoverification system increases the efficiency of clinical laboratories. But the experience-driven autoverification has shown shortages and an improvement is urgent.

Methods: The new types of autoverification rules, limit range (60 rules) and relation among tests (9 rules), were added to the original 359-rule autoverification system and the autoverification workflow was rearranged according to the priority. The ranges of limit range rules, verification range rules, relation rules and delta check rules were determined by the big data analysis of historical data from Zhongshan laboratory. The performance of the improved system was evaluated and the improvement of overall autoverification rates was assessed by a multicenter process.

Results: The improved system had a sensitivity of 96.27% and a specificity of 85.02%. The overall autoverification rates of the improved system were 43% - 93%, always higher than those of the original system, which were 36% - 81%. The customization of the improved system further increased the overall autoverification rate from 43% to 54%.

Conclusion: The data-driven range-determining strategy is feasible and practical, allowing the popularization of a well-established autoverification system. The semi-experience-driven and semi-data- driven structure is a promising structure for autoverification systems and worth further investigation.

A-005

Machine Learning Outperforms Traditional Screening and Diagnostic Tools for the Detection of Familial Hypercholesterolaemia

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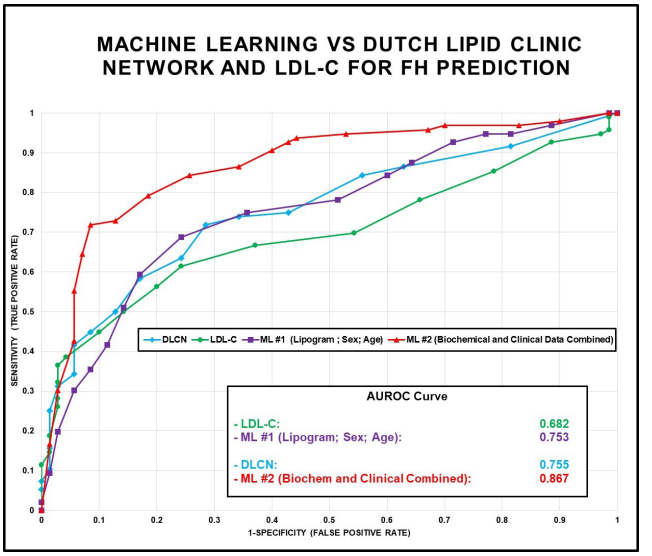
BACKGROUND: Familial hypercholesterolaemia (FH) is one of the most common monogenetic disorders worldwide, and if untreated contributes to premature cardiovascular disease, yet the majority of people with FH remain undiagnosed. The application of machine learning (ML) to biochemical and clinical data offers a unique opportunity for the detection and diagnosis of suspected FH patients. **OBJECTIVES:** To develop ML algorithms capable of detecting FH using (a) biochemical and (b) combined biochemical and clinical data with superior predictive capability compared to low density lipoprotein-cholesterol (LDL-C) cut-offs and the Dutch Lipid Clinic Network (DLCN) clinical scoring system using mutation-positive FH diagnosis as the gold standard. **METHODOLOGY:** A database consisting of biochemical, clinical, and FH-mutation status data of 555 patients clinically suspected of having FH was randomized into training (70%) and testing (30%) datasets. The training split was used to develop two distinct algorithms based on a combination of ML methodologies (random decision forest; logistic regression; and deep neural network) with BigML® software. The first algorithm (ML #1) was trained only on lipogram data, age and sex. The second algorithm (ML #2) utilized the same data as ML #1 but added clinical data (history and clinical examination). We evaluated the performance of both algorithms and the DLCN and LDL-C cut-offs on the test dataset and have reported the receiver operator characteristic (ROC) curve and calculated the area under the ROC curve (AUC) for each methodology. **RESULTS AND CONCLUSION:** As demonstrated in the graph, the ML #1 (AUC=0.753) outperformed the traditional LDL-C cut-off screening tool (AUC=0.682), despite the latter benefiting from imputed LDL-C values. ML #2, which combined biochemical and clinical data, had the best AUC (0.867), and performed markedly better than the DLCN scoring system (AUC=0.755). This study demonstrates that ML can enhance both screening for and diagnosis of FH.



A-006

Application of an Indirect Statistical Model to Support the Determination of Reference Intervals in a Large Laboratory Database

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Background: The clinical analysis of an exam happens when comparing the reference interval (RI) with the patient's result performed in the same standardized methodology.According to the College of American Pathologists (CAP) and technical note C28-A3c from the Clinical and Laboratory Standards Institute (CLSI) the responsibility is to define their own reference intervals for each laboratory, as this allows the determination of the RI regarding the assisted population.Laboratory medicine companies have large records with laboratory results and some clinical information, which can be used for data extratification, by indirect methodology, to determine their own RI, improving assistance to the result, by including factors of their determinants social and epidemiological aspects of health.Among the options: Bayesian models for computational intelligence, traditional approaches with defined control groups, data mining techniques - such as bhattacharya analysis, are dependent on the volume of data / information and are usually time-consuming processes. With that, the need arises to adapt tools to model and work with this data as an alternative to these processes, of which the R Language stands out. **Methods:** The data sources used in this experiment were eight information systems used by a Brazilian company, where each one, in particular, has a data structure and distinct tables with specific codings to catalog and qualify patient data, unique identifier that avoids duplicate entry of the same patient and created the exam categorization by code and methodology. 4,987,540 patient records were restricted to the state of Rio de Janeiro / Brazil.The transformation process used was Shine's approach that proposed normalization using a three-step approach

to data analysis, in summary: the first step identified and removed outliers, the second step allowed the definition of the underlying distribution of the other data points and the third stage stratified the data and modeled the effects of gender and age. Values between the 2.5 and 97.5 percentile limits used in the medical laboratory, suggested by Wright and Royston. When a positive asymmetry was evidenced in these frequency distributions, a logarithmic transformation of the results was made, their adequacy in a normal distribution was verified by the Shapiro test, the Kolmogorov-Smmirnov test was used to assess whether the different data sources are on equality continuous, and the 97.5 percentile for removing outliers. **Results:** The application was created to demonstrate logically and graphically the statistical completion of the pool of collected data. As an example, the valuesof the blood glucose results in venous blood, the suggested RI result is 73 to 108 mg / dL after the bhattacharya analysis suggested this RI from the normalized data. **Conclusion:** This app aims to help the laboratory make decisions about how to obtain reliable evidence of analytical and clinical validity, as well as clinical usefulness.Its results should be considered an initial part of critical thinking and an open debate for understanding the studied population.The parameters that we intend to evaluate in this study, may represent new decision limits for the Brazilian population, contributing to improve the diagnosis in our country.

A-008

Indirect vs. Direct Methods for Determining Insulin Reference Intervals among Adolescents in Rio de Janeiro, Brazil

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Background: Reference intervals (RI) are fundamental parameters to adequately interpret laboratorial results. It is advisable that each laboratory establish their own RI, however, conventional determination of RI, sampling the local population, is a costly and time-consuming process. This situation is even more critical in specific segments of the population, like adolescents and the elderly. As a consequence, is not uncommon that laboratories adopt RI cited in the literature, established for different populations and for all age strata. Despite not being the primarily recommended by the CLSI, indirect methods for RI determination are gaining popularity, especially for being faster, more economic and for allowing easier access to specific segments of the population. In this study we compared two methods (direct and indirect) for determining insulin RI among adolescents.

Methods: The direct method of RI estimation was applied to a sample of 37,892 insulin results derived from data coming from a Brazilian study among adolescents 12 to 17 yo (The ERICA Project). After the exclusion of those without self-reported health problems, or medication use, blood glucose 126uUI/ml and higher, glicated haemoglobin 6.5% and higher, elevated blood pressure, and outliers removal using Chauvenets criterion, 28,747 records were included. Bartlett test, and Kruskal-Wallis test were used to find if there were significant statistical differences by age categories and sex. The Bonferroni test was then used to separate age groups whose insulin values differences were statistically significant ($p<.05$). After grouping, RI were determined according to percentiles 2.5% and 97.5% of the distributions. For the indirect method of RI estimation, we chose to use the computerized Hofmann method. A "graphical" method intended to isolate distributions of "healthier" and "non-healthier" in the mixed sample of laboratorial results. Data for indirect RI estimation derived from laboratory insulin test results performed between 2014,January 5th to 2018, February 2nd (11,859 records), were segregated at the same age groups determined for ERICA study estimation. Data was log transformed to approach a normal distribution, a prerequisite of the methodology. Outliers were then removed using Chauvenets criterion.

Results: RI estimates by group for direct and indirect methods were, respectively: (a)Females ages 12 to 13yo (2.60-22.2uUI/ml) vs. (4.9-35.3uUI/ml); (b)Females age 14yo (2.20-21.4uUI/ml) vs. (4.4-31.5uUI/ml); (c)Females age 15yo (2.3-20.9uUI/ml) vs. (3.5-34.9uUI/ml); (d)Females ages 16 to 17yo (2.1-19.5uUI/ml) vs. (3.6-30.0uUI/ml); (e)Males ages 12 to 14yo (2.0-20.8uUI/ml) vs. (4.11-32.9uUI/ml); (f)Males age 15yo (1.88-20.2 uUI/ml) vs. (3.5-34.3uUI/ml); (g)Males ages 16 to 17yo (1.6-19.3uUI/ml) vs. (3.2-30.7uUI/ml).

Conclusion: Indirect methods RI estimates consistently presented higher values than those calculated by the direct method. Next steps in this process will be the search for possible error sources and application of other indirect estimation methods and exclusion strategies to evaluate their performance against Hofmann. Another supplemental finding in this study was that direct calculated lower RI had lower values than the commonly adopted limit (3 uUI/ml) - that is also used for the adult population. This finding, together with the statistically significant differences found by sex and age group, suggest the necessity for revision of currently used RI, to adapt for this specific age group.

A-011

Visual Identification Model Based on Fluid Biomarkers for Better Recognition of Multiple System Atrophy Cerebellar Type

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Background: Multiple system atrophy Cerebellar Type (MSA-C) is a progressive neurogenerative disorder characterized by parkinsonism, cerebellar ataxia, autonomic dysfunction and corticospinal disorders. However, as the typical clinical manifestations of MSA-C are also commonly shared with (hereditary) spinocerebellar ataxia (SCA), there can be confusions leading to misdiagnosis and prolonged consultation process even the loss of the best treatment time. Therefore, objective biomarkers properly useful for distinguishing between these two diseases would be of great help when initial clinical features are similar. Nevertheless, the research for identifying the biomarkers of MSA-C is slowly growing and few discoveries have been proved capable enough to be put into real use. In this study, we aimed to characterize possible potential biomarkers including both neurodegenerative-related and metabolic-related biomarkers mainly by Lasso Regression Analysis after which the combination of remained candidate blood biomarkers as core variables will be credited to develop a diagnostic model, assisting in the differentiation between MSA-C and SCA in the early stage of disease course as well as provide clues for further research of the disease pathogenesis. **Methods:** A total of 79 MSA-C patients and 46 SCA patients were enrolled and divided in to training set and testing set. Candidate biomarkers including basic information, metabolic and neurodegeneration related biomarkers were analyzed by univariate analysis and then selected by Least Absolute Shrinkage and Selection Operator (LASSO) algorithm. A visual and operable identification model / Web application were then generated based on the remained biomarkers by R studio and Shiny package. The receiver operating characteristic (ROC) analysis and decision curve analysis (DCA) were performed to assess the accuracy and net benefit of the model. Besides, an independent validation of 25 extra ataxia patients was also carried out to verify the model.

Results: Age of onset(AO), direct bilirubin(DBIL), aspartate aminotransferase(AST), eGFR and synuclein-alpha were selected out of 34 variables in total after the LASSO analysis. The identification model based on the 5 factors above showed good efficiency with AUC as 0.929 and 0.917 in training set and testing set respectively. The DCA plot displayed a good net benefit for this model as well. As for external independent validation, it showed that there were 13 MSA-C patients in concordance with prediction results (13/15, predictive accuracy =86.67%), and 2 patients without having been recognized by the model were then actually confirmed as MSA-C patients after the comprehensive examination (missed index=20%).

Conclusion: The visual assisting identification model based on 5 potential markers with good applicability in the differential diagnosis of MSA-C was established, hoped to help with the clinicians in the medical decisions of MSA-C.

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Clinical Lab Informatics

A-012

Using Excel and LIS Data to Dramatically Reduce Preanalytic Times

N. E. Heger, G. L. Horowitz. *Tufts Medical Center, Boston, MA*

Background: Each day blood is drawn during morning phlebotomy rounds with the goal of reporting results by 8AM in time for physician rounding in order to make decisions about patient disposition (transfer, discharge, etc.). Not having results available during rounding leads to physician frustration, wasted time, delayed bed turnover, delayed patient transfer, and possible increased length of stay. A review of our process showed that we did not consistently achieve the 8AM goal for result reporting and improvement of preanalytical workflows was needed.

Methods: Data was obtained using an SQL query from our LIS (SoftLab) to look at specimen collect time, lab receipt time, automation track receipt time, and result verified time for morning round specimens with a sodium (Chemistry) or hematocrit (Hematology) test. Data was compiled using basic pivot tables and charts in Excel and shared with all lab team members daily.

Results: At the beginning of our study ~44% of specimens did not achieve the 8:00AM cutoff. A review of LIS data indicated that improvement was needed across all phases of the preanalytic process. Staff members were prompted for input on best ways to improve the steps of specimen collection, transport, accessioning, and processing. Multiple interventions were taken during the project, including adjustment of phlebotomy rounding workflow, changes to automation track priority loading, and implementation of a real-time turnaround-time board to monitor daily progress. The 8AM report-out goal was achieved within 1 year; the process was further refined in the following 2 years.

Conclusion: The preanalytic phase of morning draw was improved without the need for increased staff, an earlier start to phlebotomy rounds, or changes to lab instrumentation/automation. Analyzing data from the LIS using Excel allowed us to identify areas for targeted adjustments to manual preanalytic workflows to improve our daily operations.

Table 1. Improvement of Preanalytic Times

	April 2016	April 2017	April 2018
Average number of draws/day by month	80	79	84
% Specimens collected by 7:15AM	83.7%	100%	100%
% Specimens transported to lab within 30 minutes of collection	65.0%	88.6%	91.7%
% Specimens delivered to automation track within 15 min of receipt in lab	47.5%	63.0%	89.3%
% Specimens Verified by 8:00AM	56.2%	100%	100%
Median time last result verified	9:36AM	7:40AM	7:40AM

A-013

Transitioning to the CKD-EPI Equation for the Calculation of Estimated GFR

J. Shi, P. Mathias, A. Hoofnagle. *University of Washington Medical Center, Seattle, WA*

Background: Chronic kidney disease (CKD) and end-stage renal disease are prevalent and growing public health issues in the United States and impose a tremendous public health burden. While directly measured GFR is considered the gold-standard assessment of kidney function, it is expensive and impractical for routine analysis as it requires administration of an exogenous marker and multiple timed urine collections or blood draws. Estimated GFR (eGFR) is therefore routinely provided as a surrogate by clinical laboratories. eGFR is a key marker for the identification and staging of CKD. It has major clinical consequences as many essential medical decisions are made based on eGFR. The MDRD is the most widely used equation in contemporary practice; however, increasing evidence has shown that the CKD-EPI equation has advantages over the MDRD equation.

Methods and results: To determine the performance of the CKD-EPI equation for our local patient population, retrospective data analysis was performed. From our data warehouse, we extracted creatinine results from 137,279 patients and associated patient demographic information. The major findings are: (1) The MDRD and CKD-EPI equations correlate well when eGFR < 60 mL/min/1.73 m^2 ($eGFR_{CKD\text{-}EPI}$ = 1.22 × $eGFR_{MDRD}$ – 8.719, R^2 = 0.98); (2) $eGFR_{CKD\text{-}EPI}$ is greater than $eGFR_{MDRD}$ when eGFR > 60 mL/min/1.73 m^2, indicating generally improved kidney function when switching to the CKD-EPI equation, which is consistent with previous studies showing that the MDRD equation tends to underestimate GFR in the general population; (3) The change in the shape of eGFR distribution is similar between black and non-black patients when switching to CKD-EPI equation, which supports the practice of not including race as a variable; (4) There is reclassification in male patients over 75 years old, in which $eGFR_{MDRD}$ >60 while $eGFR_{CKD\text{-}EPI}$ < 60 mL/min/1.73 m^2.

Conclusions: These data provide thorough evaluation of the MDRD and CKD-EPI equation on eGFR calculation from our local patient population. The data support the implementation of CKD-EPI, as well as justify the practice of removing race in the eGFR. More careful investigation in geriatric populations is warranted. This study also emphasizes the role that clinical laboratories play in changing and delineating best practices.

A-014

Creation of a Custom EPIC Dashboard of Quality Indicators Covering Multiple Laboratory Sections using SlicerDicer

S. W. Cotten. *University of North Carolina at Chapel Hill, Chapel Hill, NC*

Background: Monitoring quality indicators (QI) or key performance indicators (KPI) is an essential part of every clinical laboratory's quality management program. Updates to the 2019 College of American Pathologist's accreditation checklist now specify pre-analytic, analytic, and post-analytic quality monitoring in every laboratory section (department) for a Quality Management Program (COM.04000). The goal of this work was to develop an EPIC dashboard that utilizes SlicerDicer data analytics which can be customized for each laboratory section to meet the updated checklist requirement. Currently our medical center compiles quality indicator data from multiple data streams in a single quarterly report. The data are extracted from EPIC crystal reports, reporting workbench, manual logs and patient satisfaction surveys. In 2019, our institution launched SlicerDicer, an EPIC embedded self-reporting analytics tool for data visualization. SlicerDicer accesses data from EPIC's Cogito data warehouse (Caboodle) through a graphical user interface. Data selections are dynamically visualized and specific data queries ("sessions") can be saved by users for future reference or shared with other EPIC users.

Methods: 15 quality indicators across phlebotomy, chemistry, hematology, coagulation, blood gas, urinalysis, special chemistry, microbiology, and immunology currently included in the laboratory's quality management program were created as sessions in SlicerDicer. Additionally, four new quality indicators; hemolyzed, clotted, over citrated and QNS specimens were evaluated in SlicerDicer. Monthly data extracted from SlicerDicer was audited against the laboratory's existing quality indicator report. The difference between SlicerDicer and existing 2019 QI data were calculated for each metric by month. The ability of SlicerDicer to track quality indicators for cytogenetics, molecular microbiology, and molecular genetics was also evaluated. The individual SlicerDicer components were used to create a custom dashboard in EPIC that contained all of the specific indicators.

Results: Core lab QI metrics from for STAT hemoglobin, APTT, urinalysis and intraoperative PTH turnaround time showed an average monthly variance of 0%. STAT potassium and STAT troponin showed an average monthly variance of 1%. STAT pO2 TAT showed an average monthly variance of 2%. The QI metric for critical value notification with 30 minutes agreed within 0.025%. *Clostridium difficile* positive screen rates demonstrated an average monthly difference of 12.5%. This difference was attributed to *C. diff* antigen positive results being considered abnormal by current tracking methods but only *C. diff* toxin positive results within the SlicerDicer query. *C. diff* toxin PCR percent positive showed an average difference of -0.05%. Quantiferon TB percent positive and indeterminate rates agreed within 0.01%. Blood culture contamination rates showed an average monthly difference of +0.9%. This was considered significant as the institution's threshold is 2%. The number of QNS sweat chloride specimens agreed for all months except one; when two specimens were not brought to the lab for official cancelation in EPIC. **Conclusion:** The custom SlicerDicer dashboard is suitable for most QI metrics related to turnaround time, posi-

tive rates, specimen quality and critical value notification. Limitations exist for blood culture contamination and turnaround time monitoring greater than 240 hours which impact the cytogenetics and molecular laboratory sections.

A-016

Performance Assessment of Maximum Likelihood Ratio Method for Reference Interval Determination in Pediatric Clinical Chemistry

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Background: It is the laboratorian's responsibility to provide both accurate laboratory test results and precise reference intervals (RIs) to support the clinicians in their medical decision making. The traditional or 'direct' approach involves selecting apparently healthy individuals for sampling, usually adults, which may not always be suitable in the pediatric practice. The process of calculating RIs from a mixed population of heathy and diseased subjects is defined as the 'indirect' approach and is more applicable for obtaining pediatric RIs. The Hoffmann and Bhattacharya approaches are the two common established methods applied to mixed data but some user input (supervision) is required. Recently, a type of 'unsupervised clustering' using maximum likelihood estimation (MLE) started to gain attention for determining RIs. We wanted to assess how MLE performs in pediatric population.

Methods: All laboratory test results from January 1, 2018 to November 30, 2019 for 10 analytes (albumin, alanine aminotransferase [ALT], alkaline phosphatase [ALP], creatinine, glucose, iron, total bilirubin, total calcium, total protein and triglycerides) were extracted from our pediatric hospital's laboratory database. If appropriate for the analyte, results outside of three median absolute deviations were removed since they were considered outliers. Using R software (version 3.6.1), RIs were obtained using Hoffmann and MLE methods (mixtools package version 1.1.0) for ages <1, 1-5, 5-10, 10-15 and 15-20 years. For MLE method, normal mix model EM function using appropriate component number per analyte was utilized. For verification of the MLE approach, 20 subjects seen for an 'Encounter for routine child health examination' and tested once were randomly selected from each age group. Their results were compared to Hoffmann method's results using Kohen's kappa statistics (vcd package version 1.4-5).

Results: Table 1 shows the inter-rater reliability between two approaches. The MLE approach showed moderate reliability (Kohen's kappa coefficient [K]: 0.41-0.60) with Hoffmann method for creatinine (K: 0.50). There was substantial inter-rater reliability (K: 0.61-0.80) for albumin (K: 0.76), ALT (K: 0.79), total bilirubin (K: 0.79), glucose (K: 0.69) and total protein (K: 0.68). For ALP (K: 0.95), total calcium (K: 0.81), iron (K: 1.00) and triglycerides (K: 0.90), we observed an almost perfect inter-rater reliability (K: 0.81-1.0) between two methods.

Conclusion: Our study showed acceptable agreement between Hoffman and MLE methods. Although the Hoffmann method has been widely used in establishing RIs, it has many shortcomings such as overestimation and being influenced by non-Gaussian distribution of data. MLE and other newly developed computer intensive methods might answer these limitations and could be used in pediatric populations where the direct approach is not always suitable.

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

General Clinical Chemistry

A-017

Cell Cycle Arrest Biomarker Best Predicts AKI among Hospitalized Patients in the Central Region of Ghana

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Background: Acute kidney injury (AKI) is a major complication that contributes to morbidity and mortality. Routinely, it is diagnosed by creatinine-based guidelines and urine output, which are sub-optimal markers after injury due to renal and non-renal factors. This prospective cross-sectional study used the cell cycle arrest biomarkers (tissue inhibitor metalloproteinase 2 and insulin-like growth factor binding protein 7) and neutrophil gelatinase associated lipocalin (NGAL) to assess AKI among hospitalized patients.

Methods: We conveniently enrolled 151 in-patients at the Trauma and Specialist Hospital, Winneba, Ghana. Socio-demographic and clinical information were collected using structured questionnaires. Blood samples were collected for the estimation of serum creatinine, and AKI diagnosed and staged using the KDIGO guideline. Fresh urine samples were collected within 24 hours of admission from each patient and urinary NGAL, TIMP-2 and IGFBP-7 estimated using ELISA kits.

Results: The cell cycle arrest biomarkers and NGAL were significantly higher among participants with AKI than those without AKI. [TIMP-2]*[IGFBP-7] showed the best diagnostic performance (AUC= 0.94, CI= 0.90-0.98) followed by [IGFBP-7]*NGAL] (AUC= 0.93, CI= 0.87-0.99), with NGAL having the least (AUC=0.62, CI= 0.46-0.78). The best predictive cut-off values of the biomarkers were 39.20 for NGAL, 0.30 for [TIMP-2]*[IGFBP-7], 0.65 for [TIMP-2]*[NGAL] and 0.75 for [IGFBP-7]*[NGAL]. The cut-off for [TIMP-2]*[IGFBP-7] showed the best predictive ability (95.8% sensitivity, 77.2% specificity, 44.2% PPV and 99% NPV). The cut-off for NGAL, on the other hand, showed the least predictive ability (62.5% sensitivity, 42.5% specificity, 17.0% PPV and 85.7% NPV).

Conclusion: The cell cycle arrest biomarker [TIMP-2]*[IGFBP-7] best predicts the development of AKI, and can be used in high risk patients for early diagnosis of AKI.

Table 1: Diagnostic performance of biomarkers in predicting KDIGO AKI stage 2 and 3

Biomarkers	cut-off	AUC	Sens (%)	Spec (%)	PPV (%)	NPV (%)	P-value
TIMP-2	15.59	0.87 (0.80-0.94)	83.3	74.8	38.5	96.0	<0.001
IGFBP-7	40.68	0.91 (0.4-0.98)	91.7	80.3	46.8	98.1	<0.001
NGAL	39.20	0.62 (0.46-0.78)	62.5	42.5	17.0	85.7	0.065
[TIMP-2]* [IGFBP-7]	0.30	0.94 (0.90-0.98)	95.8	77.2	44.2	99.0	<0.001
[TIMP-2]* [NGAL]	0.65	0.88 (0.80-0.96)	83.3	78.0	41.7	96.1	<0.001
[IGFBP-7]* NGAL]	0.75	0.93 (0.87-0.99)	91.7	74.0	40.0	97.9	<0.001

AUC: Area Under Curve; Sens: Sensitivity; Spec: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value

A-022

Investigation of Diagnostic Concordance between NT-proBNP and BNP in Heart Failure Patients

P. Wang, Y. Wen, K. Chen, C. Lin. *cgmh, Taoyuan, Taiwan*

Background: Measurement of B type natriuretic peptide (BNP) and N-terminal- pro B type natriuretic peptide (NT-proBNP) is viewed as comparable in their ability to diagnose and monitor heart failure in clinical guidelines. BNP and NT-proBNP are derived from a common precursor but the clearance characteristics are different. BNP is the biologically active molecule and is cleared via specific nauretic peptide receptors; while NT-proBNP is inactive and cleared by renal excretion. The levels of the two molecules could be different. The purpose of this study was to investigate the concordance between NT-proBNP and BNP.

Methods: The study population consisted of 275 patients, average age was 66 years old, having routine physician simultaneously ordered BNP and NT-proBNP between July 2017 and December 2019. Both BNP and NT-proBNP were measured on EDTA plasma. BNP were measured by two-site immunoenzymatic assay on Beckman Coulter with Alere Quidel Triage reagent. NT-proBNP was measured by electrochemiluminescence immunoassay on a Cobas e411 automated chemistry analyzer. Cutoffs for BNP is 100 pg/mL and NT-proBNP is 450 (< 50 years), 900 (50-75 years), 1800 (> 75 years) pg/mL to rule out or rule in heart failure.

Results: Of the 275 patients, 222 patients (81%) had diagnostically concordant BNP and NT-proBNP. To investigate the 53 discordant cases, 16 patients were diagnosed as heart failure. There 3 heart failure patients with normal BNP, and 13 patients with normal NT-proBNP evaluated by the age specific cutoffs. Of the 13 cases, 4 were prescribed with Entresto, 6 are older than age 75. OF the 37 non-heart failure patients, 3 are with elevated NT-proBNP and 34 are with elevated BNP. One of the 34 patients was prescribed with Entresto, and 21 are older than age 75. It is well known that BNP level in patients prescribed with Entresto will be elevated. The falsely normal BNP in heart failure patient and falsely elevated NT-proBNP non heart failure patients could be due to the short half-life of BNP . For the unknown reason discordant cases, we are not able to get the renal function evaluation but we found two thirds of the cases are older than age 75.

Conclusion: We found good diagnostic concordance between NT-proBNP and BNP for ruling in and ruling out heart failure.

A-023

Usefullness of the Urine Dipstick Albumin to Creatinine Ratio and Dipstick Proteinuria for Screening of Chronic Kidney Disease in Health Checkups

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Backgroud: Chronic kidney disease (CKD) is getting an attention as a worldwide health problem related to premature death and worse quality of life. Albuminuria or proteinuria are frequently used marker of kidney damage in clinical practice. Semi-quantitative evaluation by dipstick has become available to detect urinary albumin-to-creatinine ratio (ACR) and proteinuria simultaneously in spot urine sample. The aim of this study was to evaluate the usefulness of urinary ACR and dipstick proteinuria for CKD screening in health checkups.

Methods: Subjects of this cross sectional study were 88,479 health examinees underwent health checkup including urinary ACR, proteinuria, and urinalysis by dipstick (UC-3500 urine chemistry analyzer, Sysmex, Kobe, Japan) at 16 health promotion centers in 13 Korean cities between January 2018 and Septemberr 2019. Albuminuria was defined using an ACR cutoff of 30 mg/g or 300 mg/g. Proteinuria was defined using an protein -to-reatinine ratio (PCR) cutoff of 150 mg/g or 500 mg/g and a urine dipstick cutoff of trace or 1+. CKD was defined as a urinary ACR≥30 mg/g or estimated glomerular filtration rate (eGFR)<60 mL/min/1.73 m2. CKD risk categories were created using a combination of eGFR and urinary ACR.

Results: The prevalence of ACR 30-300 mg/g and >300 mg/g vs dipstick PCR 150-500 mg/g (≥trace) and >500 mg/g (≥1+) were 9.5% and 1.9% vs 5.2% (2.9%) and 1.4% (5.5%), The prevalence of ≥moderately increased CKD risk using ACR (≥30 mg/g), the protein dipstick (≥trace), and PCR (≥150 mg/g) were 16.37%, 12.71 %, and 11.96%, respectively. The concordance rates between the ACR-based and PCR or protein dipstick-based CKD risk category were 92.06 % (κ values: 0.683) and 88.76% (κ values: 0.567), respectively. ACR-based CKD risk categories were classified into lower risk categories, when those were grouped based on the protein dipstick results. The underestimated percents of CKD risk using the dipstick cutoff of trace and 1+ proteinuria were 6.09% and 7.81 % of the study subjects, respectively. The sensitivity, specificity, and area under the curve (AUC) for ≥moderately increased CKD risk were 69.4%, 100% and 0.847 for ACR (≥30mg/g), 33.1%, 98.6% and 0.658 for PCR (≥150mg/g), 33.8%, 97.7% and 0.659 for proteinuria (≥trace), and 25.8%, 98.5% and 0.621 for proteinuria (≥1+), respectively.

Conclusion: Urinary dipstick proteinuria had lower sensitivity and specificity for ≥moderately increased CKD risk compared to dipstick ACR. CKD risk category using urine dipstick proteinuria was underestimated compared to ACR-based CKD risk category. This suggests that ACR-based CKD screening is needed in health checkups.

Keywords: Chronic kidney disease, dipstick albumin-to-creatinine ratio, dipstick proteinuria

A-024

Monoclonal Immunoglobulin Interference in Beckman Coulter AU5800

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Background: Monoclonal immunoglobulin (M protein) is mainly secreted by a large number of plasma cells with abnormal clonogenic hyperplasia. M protein is commonly seen in monoclonal immunoglobulin proliferative diseases, such as multiple myeloma (MM), Waldenstrom macroglobulinemia (VM), heavy chain disease, light chain disease, etc. M protein has unique physical, chemical and immunological properties, and has been reported to interfere with many clinical tests. The purpose of this study was to evaluate the interference of monoclonal immunoglobulin in Beckman Coulter chemistry analyzer AU5800, and to propose solutions for removing interference of M protein.

Methods: Comparison of 40 cases of different concentrations and types of M protein serum specimens and 6 cases of apparent healthy person serum specimens, quantitative detection of GGT, LDH, ALP, TBil, DBil, PA, Na, Ca, P, Glu, UA, Cr, BUN, HDL-C, hs-CRP respectively in Beckman Coulter AU5800 and Johnson & Johnson VITROS 5600. The deviation between the different detection systems were calculated. The reaction curves of M protein positive serum samples on Beckman Coulter AU5800 were observed to identify the abnormal response curves. For the samples with abnormal reaction curve, different methods were used to try to eliminate the interference.

Results: The quantitative results of TBil, DBil, Na, Ca, P, Glu, UA, Cr, BUN and HDL-C were all lower than the national inter-laboratory quality assessment (EQA) analysis standard in AU5800 and dry chemistry. For the ALP test, the dry chemical and enzymatic quantitative results of M protein positive serum samples were significantly higher than those of normal human serum samples. In LDH test, the quantitative results of dry chemical test methods of all samples were higher than those of enzymatic test methods, with methodological differences. The abnormal response curve are observed in P, UA, and PA, IgG, IgA and IgM. Dilution can eliminate interference to P. For PA dilution can also be used as the first choice to eliminate interference. For UA the best method is to change the detection system or precipitate M protein before detection.

Conclusion: M protein interference induced the response curve of Beckman Coulter AU5800 was abnormal or the result was biased, but it was related to the concentration and type of M protein. Dilution can reduce some interference, or change of the detection system to eliminate M protein interference.

A-026

A Posteriori Reference Interval Calculation using Local Patient Data as an Adjunct to Reference Interval Verification and Transference

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OBJECTIVES: Defining reference intervals through recruitment of healthy individuals is a resource-intensive task for clinical laboratories and may become impractical when the project scope is large (e.g., when simultaneously defining reference intervals for many tests). In such cases, transference of previously defined reference intervals using method comparison studies is an established approach but in practice may propagate reference intervals that have not been verified in the local population. Here, we sought to assess the potential value of local patient data and *a posteriori* reference interval calculation as an adjunct to established methods for defining reference intervals. METHODS: De-identified laboratory data from 115,503 presumed healthy local outpatients were obtained and used to calculate reference intervals for 16 common clinical chemistry tests. The presumption of health was based exclusively on patient visit location, with only outpatient general practice and laboratory visits being included. Data were partitioned by sex in cases where this information was available. Reference intervals were calculated using nonparametric and robust methods with and without outlier removal (Horn method). RESULTS: Sex-based partitions were created for 4 of the 16 tests studied, resulting in 20 total partitions from which reference intervals were calculated. At least 2500 patients were included in all but one partition; no partition included fewer than 700 patients. Reference intervals calculated using the nonparametric method after Horn removal of outliers agreed best with established values. Using this method, lower (2.5th percentile) and upper (97.5th percentile) reference limits were within total allowable error (TEa) of established values for 80% (16/20) and 85% (17/20) of partitions, respectively. Of the 16 tests studied, 12 showed no significant difference between nonparametrically calculated and established reference intervals: albumin, anion gap, AST, bicarbonate, BUN, total bilirubin, total calcium, chloride, creatinine, total protein, sodium, and free thyroxine. Four tests showed significant, though not necessarily clinically impactful, differences between nonparametrically calculated and established reference intervals; ALP, ALT, potassium, and TSH. Lower reference limits calculated using the robust method deviated significantly from established values in 12 of 20 partitions, particularly when the population median value was closer to the assay's limit of detection. Upper reference limits calculated using the robust method were more accurate, and 18 of 20 partitions (14 of 16 tests) showed agreement between calculated and established values within TEa. CONCLUSIONS: Simple inclusion/exclusion criteria identified a local cohort of patients in which nonparametrically calculated *a posteriori* reference intervals agreed with established values for 12 of 16 common clinical chemistry tests. The robust method appeared less suitable for *a posteriori* reference interval calculation, as lower reference limits often deviated significantly from established values. The patient selection and reference interval calculation methods described are likely accessible to most clinical laboratories and provide a means to assess the suitability of established values for the local population, potentially identifying tests for which more detailed study (e.g., reference interval establishment) would be beneficial. Given inherent challenges in establishing the health of reference individuals using statistical methods alone, these results do not suggest that *a posteriori* methods can replace direct sampling techniques for reference interval establishment.

A-029

False Positive Urine Protein Results by Reagent-Strip Method of Urinalysis

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Introduction: The reagent-strip testing for protein included in urinalysis is a rapid and inexpensive method for screening proteinuria. However, the method can produce false positive or false negative results. We noticed the tendency of false positive urinary protein results by Beckman-Coulter Iris iChemVelocity and performed confirmatory test in the past two years. We present the false positive rate of protein by iChemVelocity and by Arkray Aution Max AX-4030 (AutionAX-4030) using sulfosalicylic acid (SSA) as a confirmatory test. **Methods:** 5056 Urine samples collected in both in-patient and out-patient settings were tested on the same day of collection by iChemVelocity. Any sample with a 1+ or higher protein result was also tested by SSA method. 425 urine samples with positive protein results by the AutionAX-4030 were also tested using SSA method. The false positive rate for protein was obtained for both iChemVelocity and AutionAX-4030. **Results:** The comparison between iChemVelocity and SSA can be seen in Table 1. About 599 samples (11.9%) had concordant results by both methods. The false positive rate for protein was 78% (3815 with 1+, 128 with 2+, and 2 with 3+) by iChemVelocity as compared to the results by SSA method. The concordant rate for inpatient and outpatient settings were not significantly different (9.3% *versus* 10.6%, Mann-Whitney *p*-value = 0.7518). The false positive rate for protein by AutionAX-4030 was 11.3% when compared to SSA. There were 193 (45.4%) concordant results, 180 (42.3%) results differing by 1 grade. **Conclusions:** The false positive rate for protein was significantly higher with iChemVelocity (78%) than with AutionAX-4030 (11.3%). We therefore recommend using confirmatory test to determine the specificity and sensitivity of a reagent-strip testing for protein when validating a method for urinalysis.

Table 1 Comparison of positive protein results by iChemVelocity and SSA methods

		iChemVelocity			
		Negative	**1+**	**2+**	**3+**
SSA	**Negative**	0	3815	128	2
	Trace	1	99	23	1
	1+	0	305	300	5
	2+	0	15	228	40
	3+	0	3	25	66

A-033

Evaluation of phospho-Tau, total-Tau, and Neurofilament Light Chain in CSF for the Diagnosis of Sporadic Creutzfeldt-Jakob Disease

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BACKGROUND: While the definitive diagnosis of sporadic Creutzfeldt–Jakob disease (CJD) requires a postmortem brain biopsy; the antemortem diagnosis of CJD is challenging and currently made by a combination of clinical criteria and supporting tests such as electroencephalography, magnetic resonance imaging, and cerebrospinal fluid (CSF) studies. The real-time quaking-induced conversion (RT-QuIC) assay, based on the fluorescence detection of misfolded prion protein in CSF, is included in the CDC's most current diagnosis criteria of probable CJD due to its high sensitivity (97%) and specificity (100%). In the US, the RT-QuIC assay is performed as part of the Prion disease panel for CJD in the National Prion Disease Pathology Surveillance Center (NPDPSC, Cleveland, OH), and provides an estimated probability of CJD by combining the results of RT-QuIC and the CSF biomarkers, 14-3-3 and total Tau. Although RT-QuIC testing provides a high diagnostic accuracy for CJD, its complexity, scarce availability, and long turnaround time, prompts the search for more accessible biomarkers. The objective of this study was to evaluate various CSF biomarkers of neuronal damage: total-Tau (t-Tau), phospho-Tau (p-Tau), neurofilament light chain (NF-L), and compare their performance to the NPDPSC Prion disease panel. **METHODS:** Residual CSF specimens from 33 Mayo Clinic patients in whom the NPDPSC assay was ordered clinically were included in this study. t-Tau and p-Tau were measured using the Roche Elecsys assays (Roche Diagnostics, Inc., IN) and NF-L was measured using the Simoa® analyzer (Quanterix Corp., MA). Classification of probable CJD (pCJD) and non-CJD was based on the results of NPDPSC assay panel. Patients were classified as pCJD (n=8) when a 98% probability for CJD was reported based on the results of NPDPSC assay panel. If the probability of CJD was reported as 5% or less based on the results of NPDPSC assay panel, the subjects were classified as non-CJD (n=25). Markers were compared in the pCJD and non-CJD groups and cut-offs that provided the best concordances with the NPDPSC assay classification were evaluated. Statistical significance was established at $p \leq 0.05$ using the non-parametric Wilcoxon-Mann-Whitney test. **RESULTS:** t-Tau and NF-L concentrations were significantly increased in the pCJD group; t-Tau (pg/mL) (mean±SEM) (pCJD:1104±107 *vs* non-CJD:246±49, p<0.001) and NF-L (pg/mL) (mean±SEM) (38929±6026 *vs* 13901±2382, p=0.008). p-Tau concentrations were not significantly different (p=0.2528) between the pCJD (26.5±5.3 pg/mL) and non-CJD (22.4±3.4 pg/mL) groups. The p-Tau/t-Tau ratio was significantly decreased in the pCJD group compared to the non-CJD group (0.024±0.004 *vs* 0.100±0.003, p<0.001). At optimal cut-points, the positive (pos) and negative (neg) concordances with the NPDPSC assay's CJD classification were as follows: t-Tau (510 pg/mL) 78% pos and 96% neg; p-Tau/t-tau (0.042) 100% pos and 96% neg ; and NF-L (23800 pg/mL) 50% pos and 90% neg. **CONCLUSION:** In this pilot study, the use of the p-Tau/t-Tau ratio provided excellent discrimination against the NPDPSC non-CJD and pCJD classification. However, further studies with a larger sample size and correlation with the pathological diagnosis is required for an accurate evaluation of the diagnostic utility of these markers.

A-038

Development of B.R.A.H.M.S Procalcitonin Assay using LOCI Technology on the Dimension EXL Integrated Chemistry System

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Background: Siemens is developing a B.R.A.H.M.S Procalcitonin (PCT) assay utilizing LOCI® technology on the Dimension® EXL™ integrated chemistry system. The Dimension EXL system incorporates multiple detection technologies which enables high-sensitivity immunoassays.

Method: The Dimension EXL PCT assay is a homogeneous sandwich chemiluminescent immunoassay based on LOCI technology. The assay measures the PCT concentration in both serum and plasma. A patient sample is incubated with a biotinylated antibody to form a complex. Dyed bead reagents (Chemibeads) coated with two additional antibodies are added to form a bead-PCT-biotinylated antibody sandwich. Streptavidin-coated bead reagents (Sensibeads) containing a photosensitive dye are added and bind to the biotin to form a bead-aggregated immunocomplex. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from the Sensibeads, which triggers a chemiluminescent reaction in the Chemibeads. The resulting signal is measured at 612 nm and is proportional to the concentration of PCT in the sample. Calibrator values are traceable to the B.R.A.H.M.S Kryptor values.

Results: Time to first result is 23.5 minutes. The assay requires 5 µL of serum or plasma and is linear from 0.02 to 50 ng/mL. Reproducibility was assessed using eight patient pools from 0.02 to 50 ng/mL of PCT. Repeatability CVs ranged from 1.7 to 5.0%. Within-lab CVs ranged from 2.4 to 5.3%. Patient sample comparison between this method and B.R.A.H.M.S PCT sensitive Kryptor method produced the following Passing-Bablok regression statistics: slope = 1.02, intercept = -0.04 ng/mL, r^2 = 0.9609, and n = 88 over a concentration range of 0.06-49.71 ng/mL. When focusing on the Dimension EXL PCT assay range below 2.0 ng/mL, the Passing-Bablok regression statistics were as follows: slope = 0.97, intercept = -0.03 ng/mL, r^2 = 0.973, and n = 47 over a concentration range of 0.06-1.91 ng/mL. No significant interference bias (<10%) was observed in the presence of the physiological interferences cholesterol, triglycerides, lipemia, bilirubin or hemoglobin at PCT concentrations of 0.25 and 2.0ng/mL. At these same two levels of PCT, less than 10% bias interference was measured with samples containing 1200 ng/mL biotin. Common cross-reactants (human, eel and salmon calcitonin, human katacalcin, alpha-CGRP and beta-CGRP) were also tested and less than 10% cross-reactivity was measured.

Conclusions: The Dimension EXL B.R.A.H.M.S PCT assay demonstrates acceptable precision, accuracy, and turnaround time for the measurement of PCT in serum and plasma.

**Under development. Not available for sale.*

A-039

Vitamin B_6 Deficiency is Associated with Decreased Measured Concentrations of Alanine Aminotransferase and Increased Measured Concentrations of Alkaline Phosphatase

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Background. Pyridoxal 5′-phosphate (PLP), the biologically active form of vitamin B_6 (VB_6), is required as a cofactor for activity of the liver aminotransferase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Alkaline phosphatase (ALP) catalyzes dephosphorylation of PLP to the metabolite 4-pyridoxic acid, which is excreted. Considering the observed high prevalence of VB_6 deficiency (20%) and excess (17%) in the US population (2019 Online Abstract Archive, A-208), we examined association between concentrations of PLP, ALT, AST, and ALP, as well as age and between-sex differences. **Methods.** We reviewed historic data for PLP, ALT, AST and ALP concentrations measured in consecutive routine patient samples (n=769) submitted for clinical testing. Specimens were from adults aged 18-94 y; median (mean) ages were 51 (50) years. Among the analysed samples, 67.0% were from adults (18-59 y), 30.4% from older adults (60-79 y) and 2.6% from elderly (>79 y). More specimens were received from women (59.6%) than from men (40.4%). PLP was measured using a validated LC-MS/MS method; ALT, AST and ALP were measured using Architect 4000 or Architect 8000 analysers (Abbott). The enzyme assays did not include addition of PLP to the samples as part of the procedure. The imprecision of all utilized assays was <10%. The reference interval (RI) for PLP was 20-125 nmol/L; RIs for ALT, AST, and ALP were 5-60 U/L, 15-40 U/L, and 38-126 U/L, respectively. **Results.** Median (mean) PLP concentrations in samples from adults, older adults and elderly were 82 (56), 98 (51) and 87 (55) nmol/L, respectively. PLP concentrations were below the RI in 16.0% of the samples and above the RI in 20.8%. Multivariate analysis showed significant positive association between concentrations of PLP and the enzymes (ρ=0.090, p=0.013 for ALT; ρ=0.169, p<0.0001 for AST; and ρ=-0.273, p<0.0001 for ALP), and between age and concentrations of AST (ρ=0.111, p=0.0021) and ALP (ρ=0.154, p<0.0001). Statistically significant differences were observed between men and women in the distribution of concentrations of PLP (p=0.045) and the enzymes (ALT, AST and ALP all p<0.0001). Statistically significant positive correlation (log-log transformed values) was observed between PLP concentrations with measured ALT (R^2=0.005, p=0.046) and AST (R^2=0.010, p=0.0049); statistically significant negative correlation was observed with concentrations of ALP (R^2=0.081, p<0.0001). No ALT concentrations above the upper limit of the RI were observed for severely VB_6 deficient samples (PLP <10 nmol/L); the positivity rate in samples with PLP concentrations >10 nmol/L ranged between 6.3-9.4%.The positivity rate for AST was independent of PLP concentration (7.8-12.5%). The positivity rate for ALP in severely VB_6 deficient samples (PLP <10 nmol/L) was 34.4%; and decreased progressively as PLP concentrations increased (18.9%, 10.8%, 7.8%, 5.3, 6.6% and 0%, in groups with PLP concentrations ranging from 10-19, 20-39, 40-59, 60-124, 125-249, and >250 nmol/L, respectively).**Conclusions.** We observed significant association between PLP concentration and concentrations of ALT, AST and ALP. Among specimens severely deficient in VB_6, no samples were positive for ALT, while the positivity rate of ALP in this group was the highest; positivity for AST was not associated with VB_6 status.

A-040

Analytical and Clinical Performance of Roche Elecsys® Alzheimer's Disease Biomarkers in Cerebrospinal Fluid

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Background: Alzheimer's disease is characterized by the formation of extracellular amyloid plaques composed of beta amyloid proteins as well as intracellular neurofibrillary tangles consisting of tau proteins in the brain. Measurement of β-amyloid (1-42) (Aβ42), phospho-Tau (181P) (p-Tau), and total-Tau (t-Tau) in cerebrospinal fluid (CSF) can be potentially utilized to differentiate Alzheimer's disease from other neurodegenerative processes. **Objective:** Establish the analytical and clinical performance characteristics of the Roche Elecsys immunoassays (Roche Diagnostics) for the quantitation of Aβ42, p-Tau, and t-Tau in CSF. **Methods:** The Roche Elecsys Aβ42, p-Tau, and t-Tau assays were performed on the Roche Cobas e601 per manufacturer instructions. The analytical performance characteristics of the assay were established using residual CSF samples collected at Mayo Clinic which were then poured into Sarstedt low bind polypropylene tubes. Studies were performed using two (Aβ42 and p-Tau) or one reagent lot (t-Tau). Analytical validation studies included stability, imprecision, accuracy, analytical measuring range (AMR), dilutions, limit of detection (LOD), lower limit of quantitation (LOQ), carryover, effect of antigen excess (hook-effect), and interfering substances. For evaluation of clinical performance, the three biomarkers were measured in a cohort of clinically characterized samples (n=161). **Results:** All biomarkers were stable in CSF up to 12 hours ambient, 14 days at 4°C, and 30 days at -20°C. Analytical performance characteristics for Aβ42, p-Tau, and t-Tau in CSF, respectively, are as follows: Intra-assay imprecision (concentration ranges 310-1,184 pg/mL, 8.7-104 pg/mL, and 106-1,081 pg/mL) ranged from 2.4-3.8%, 0.8-2.5%, and 0.6-1.9%. Inter-assay imprecision (concentration ranges 248-1,086 pg/mL, 9.6-45.4 pg/mL, and 115-661 pg/mL) ranged from 2.4-6.6%, 1.5-18.1%, and 1.1-4.1%. Accuracy was acceptable with average recoveries of 99%, 96%, and 92% of the expected concentrations. The AMRs were: 250-1,700 pg/mL, 8-120 pg/mL, and 80-1,300 pg/mL. Dilution studies demonstrated a lack of suitable diluent for all three analytes. The assays LOD (LOQ) were 27.6 (250 pg/mL), 2.1 (8.0 pg/mL), and 5.0 (80 pg/mL). No carryover was observed up to 146,128 pg/mL, 1,217 pg/mL, and 11,276 pg/mL. No hook-effect was observed up to 146,128 pg/mL, 380 pg/mL, and 4,267 pg/mL. Hemoglobin, bilirubin, and biotin were acceptable up to 500 mg/dL, 25 mg/dL, and 30 ng/mL, respectively. Mean (median) concentrations for cognitively unimpaired (CU) (n=119), mild cognitive impairment (MCI) (n=24), and Alzheimer's disease (AD) dementia (n=18) patients, respectively, were as follows: Aβ42: 1,300 (1,368 pg/mL), 1,137 (1,078 pg/mL), and 563 (544 pg/mL); p-Tau: 18.4 (15.7 pg/mL), 23.0 (20.8 pg/mL), and 35.0 (33.4 pg/mL); t-Tau: 210 (188 pg/mL), 253 (250 pg/mL), and 360 (334 pg/mL). Using the p-Tau/Aβ42 ratio with a previously defined cutoff of >0.023, 20% of CU, 41% of MCI, and 100% of AD dementia patients were classified as positive. **Conclusion:** The observed analytical performance of the Roche Elecsys immunoassays for Aβ42, p-Tau, and t-Tau appeared robust and suitable for clinical laboratory utilization. Clinical performance using previously established internal cutoffs for the p-Tau/Aβ42 ratio was deemed acceptable.

A-041

Concurrent Bisalbuminemia and Hypoalbuminemia: A Case Report

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Background: Bisalbuminemia is a uncommon finding that can be detected by serum protein electrophoresis (SPEP) based on the bicuspid patterns in the albumin fraction. The cause of bisalbuminemia can be inherited due to gene mutation or can be acquired and transient due to couple pathological states. The prevalence of bisalbuminemia in Taiwan is unknown and probably infrequent. Based on the literature review, it is unknown whether hypoalbuminemia will be associated with a decrease of alloalbumins.

Case Report: We report the case of a 20-year-old man who has systemic lupus erythematosus (SLE) and concurrent hypoalbuminemia and bisalbuminemia. The initial presentations of SLE included malar rash, photosensitivity, fever, and arthralgia involving knee joints. The disease status soon progressed and the patient developed psychotic symptoms. Laboratory examinations then revealed pancytopenia, along with elevated antinuclear and anti-Smith antibodies, and positive diluted Russel Viper Venom Time (dRVVT) test. SPEP was examined for diagnostic workup, and decreased albumin fraction (43.6%) was found. A tiny peak was also found following the albumin fraction, and was recognized as increased alpha1 globulins (16%). Meanwhile, the serum albumin level was 3.1 g/dL, when SPEP was performed. After the patient received therapies, the activity of SLE was well controlled, and the patient recovered well. The SPEP was followed about 1 year later, when the patient had serum albumin level of 5.0 g/dL. Interestingly, the SPEP results showed bisalbuminemia and albumin fraction was 64.3%. We compared the two SPEP results, and found the tiny peak recognized as alpha1 globulins in the initial SPEP was slow- migrating albumin. The SPEP was performed using a 1:1 mix of patient serum with normal serum. The results showed that alloalbumin fraction decreased from 25.1% to 14.7%.

Conclusion: We demonstrate that hypoalbuminemia can lead to failed detection of bisalbuminemia by SPEP, and reveal the different dynamics of normal albumin and alloalbumin in disease states.

A-042

Reference Range Establishment/Validation Challenges. "One Size Does Not Fit All"

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Background:

The HealthCare System implemented an automated chemistry / immunoassay systems as well as instrument standardization across three sites. Standard practices were followed for the validation of the manufacturer's suggested reference ranges. Approximately three to six months after implementation physician feedback began to grow that a few analytes reference ranges were not consistent with clinical presentation.

Method:

Initially, CLSI C28-A3C *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory* was used as a guide to validate the manufacture's provided ranges. This provided the health system objective data confirming the ranges that were implemented as we went live with the new systems. Blood specimens were obtained from lab personnel accompanied by a questionnaire and an informed consent. The reference range study included 67 serum samples from apparently healthy adults and the results were analyzed using EP Evaluator (Data Innovations).

Results:

As feedback began to build that data was not consistent with clinical presentation, which began to lead to unnecessary additional confirmatory testing. Novel approach was taken with the use of data analytics tools to pull patient data segmented by sex, age and ICD-10 indications. We ultimately focused on ICD-10 code Z00.00 *"Encounter for general adult medical examination without abnormal findings*". This approach provided us an objective independent view of "normal data" of several key tests.

Conclusion:

Established recommendation and process provide a starting point in the establishment of reference ranges, but for some unique analytes subsequent data analytics review post go-live are key to establishing a robust reference range that is more appropriate for the laboratory's patient population and will provide data consistent with clinical presentation.

A-043

Performance Evaluation of the ADVIA Chemistry Enzymatic Hemoglobin A1c_E Calibrator

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Background: The purpose of the investigation was to evaluate the commutability and uncertainty of the Enzymatic Hemoglobin A1c_E Calibrator on the ADVIA® Chemistry Systems. The ADVIA Chemistry A1c_E assay is an enzymatic method that specifically measures N-terminal fructosyl dipeptides on the beta chain of HbA1c. 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 ADVIA Chemistry A1c_E reagent 1 [R1] + 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 478/805 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. The ADVIA Chemistry A1c_E assay incorporates a turbidity normalization mechanism (cHb_E) that is measured at 884 nm to effectively remove any sample turbidity that could affect the tHb_E measurement.**Method:** Performance testing included a commutability study and uncertainty estimation. The commutability study used a Deming regression and 76 whole-blood samples to compare the ADVIA Chemistry Enzymatic Hemoglobin A1c Assay cali-

brator versus the designated comparison method (DCM) performed by the National Glycohemoglobin Standardization Program (NGSP). The estimation of uncertainty of the ADVIA Chemistry A1c_E calibrator's assigned values was calculated using real-time data and manufacturing process review for %HbA1c and mmol/mol HbA1c, with uncertainty reported in percent. **Results:** The evaluation of commutability of the ADVIA Chemistry A1c_E calibrator using 76 whole-blood samples tested within the NGSP network yielded the following regression equation: ADVIA Chemistry A1c_E = 1.013 [NGSP] - 0.04 HbA1c (r = 0.987)The product calibrators fall within the 95% confidence interval of Deming regression.The %HbA1c values assigned to the two-level product calibrators have an estimated 0.12-0.24 %HbA1c (0.76 to 2.16 mmol/mol) expanded total uncertainty in percent (k = 2). **Conclusion:** ADVIA Chemistry Systems Enzymatic Hemoglobin A1c Assay calibrator demonstrated commutability to the NGSP DCM. The expanded total uncertainty from the manufacturing process is sufficient to support its intended use.*Product availability may vary from country to country and is subject to varying regulatory requirements.© Siemens Healthcare Diagnostics Inc., 2020

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A-046

Precision Study with Oral Epithelial Cells on AUTION EYE (TM) AI-4510 Urine Sediment Analyzer

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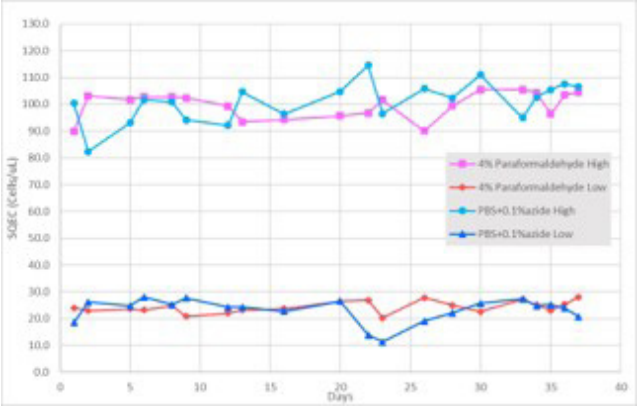
Background and Objective: ARKRAY, Inc AUTION EYE Fully Automated Urine Particle Analyzer (AI-4510) is an automated urine particle analyzer for *in vitro* diagnostic use which analyzes twelve (12) parameters in urine. This device (not for sale in USA) uses digital cameras to capture images of the formed elements as the sample passes through a flow cell. The captured images are examined using proprietary software that can classify and count the number of formed elements. A precision study was conducted to determine the variability of squamous epithelial cell (SQEC) measurements by AI-4510.

Methodology: A precision study was performed per CLSI EP05-A3 *Evaluation of Precision of Quantitative Measurement Procedures*. Human oral epithelial cells were collected from the inner lining of cheeks of normal donors. The stability of the cells was examined during the 20-day study using either a fixative or a chemical preservative. The oral SQECs were tested at concentrations of 100 and 25 cells/µL for 20 non-consecutive days. Samples were run in triplicate each day.

Validation: The precision of oral SQECs fixed with 4% paraformaldehyde had %CVs of 9.4 and 13.4 while the unfixed cells in 0.1% azide had %CVs of 10.3 and 21.7 at cell concentrations of 100 and 25/µL, respectively (Figure 1). Bacterial contamination was also assessed, and the fixed cells had a low amount of bacterial contamination (mean value of 2 and 0.6 cells/µL) at the two SQEC concentrations whereas the unfixed cells had higher mean bacterial counts (9.7 and 2.3/µL).

Conclusion: In the absence of commercial control solutions that contain epithelial cells, a novel preparation of epithelial cells from human cheeks, fixed in 4% paraformaldehyde, can be used for long term precision study on the AI-4510 urine sediment analyzer. Precision for fixed oral SQECs at 100 and 25 cells/µL was 9.4 and 13.4 %CV, respectively.

Figure 1:



A-047

Prevalence of Samples with Biotin Concentrations Capable of Causing Potential Immunoassay Interference and Effectiveness of a Biotin-Removal Reagent

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Background: The U.S. Food and Drug Administration (FDA) issued a safety communication on biotin interference with immunoassay results in 2017 and again in 2019. Many immunoassays utilize biotinylated reagents. Depending on the manufacturer and immunoassay utilized, exogenous biotin may falsely increase or decrease test results. Consequences of such interference may alter the course of action in a patient's diagnosis or treatment. The primary objective of this study was to determine the prevalence of samples that contain biotin at quantities capable of causing immunoassay interference. The secondary objective was to evaluate the efficacy of a pretreatment reagent designed to remove biotin from patient samples. **Methods:** 397 residual, de-identified serum or plasma samples obtained from patients between 18 and 75 years of age were randomly selected. Of these, 234 (59%) were from outpatients and 163 (41%) were from patients sen in an emergency department. Each sample was qualitatively tested for the presence of biotin (>=15 ng/mL) using VeraTest Biotin™ (Veravas, Inc., Oakdale, MN). Positive samples were treated with VeraPrep Biotin™ (Veravas, Inc.) per manufacturer instructions. Aliquots of pre- and post-treatment samples were analyzed for thyroglobulin (Tg) and CA-125 using a Siemens Immulite (Siemens Healthcare Diagnostics, Tarrytown, NY), and free T3 (fT3) using a Siemens Vista, methods identified by the manufacturer to exhibit potential biotin interference. The study was approved by the Advarra Institutional Review Board.

Results: Please see table for results. Bold font indicates a difference between pre and post test results that exceeds total allowable error. **Conclusion:** There is a low prevalence of samples with biotin >=15 ng/mL in the population tested. Nine of the 11 samples had at least one test result adversely affected by biotin. VeraPrep Biotin appears to be effective at removing biotin interference.

Number of samples positive for biotin: 11/397 (2.8%)						
Sample ID	Tg (ng/mL)		CA-125 (U/mL)		fT3 (pg/mL)	
	Pre	Post	Pre	Post	Pre	Post
B-006	9.6	14	7.1	4.8	2.8	2.8
B-018	3.6	26.9	13.3	8.2	4.1	2.5
B-053	17.1	14.6	5.6	4.9	3.0	3.4
B-142	7.7	19.7	12.4	8.1	2.8	2.6
B-183	3.4	18.2	244	167	4.9	3.6
B-212	9.4	43.8	21	13.9	3.3	2.4
B-217	1.2	1.3	4.9	4.6	2.5	2.8
ER-019	8.2	8.5	9.5	10.4	2.2	2.3
ER-021	20.5	19.4	185	196	1.9	1.9
ER-066	6.8	13	5.7	8.0	3.7	2.2
ER-141	QNS	QNS	6.3	6.6	1.9	2.0
p	0.037		0.31		0.24	

A-048

Verification of Sex and Age Partitioned Reference Intervals for CSF Total Protein on the Siemens Advia

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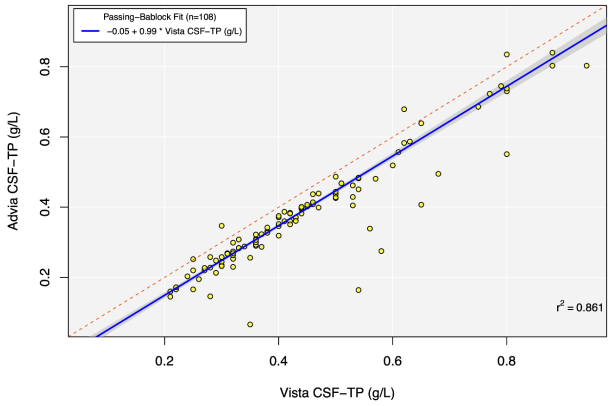
Background: To interpret laboratory results, it is essential to have reference intervals. Reference intervals for cerebrospinal fluid total protein (CSF-TP) are commonly derived from old literature because of the invasive nature of CSF sampling. The objective of this study was to transfer CSF-TP reference intervals between two different Siemens methods, the Vista and Advia.

Methods: CSF samples from 252 adults were compared between the Siemens Vista 1500 and Advia 1800. Both methods use pyrogallol red and sodium molybdate. Excluded were duplicates from the same patient, low volume samples (<250 uL), and

results below the measuring limit of either method (128 exclusions total). Passing-Bablock regression was used to estimate the slope and intercept. We used values that were within the range the source reference interval (~0.1-1.0 g/L); specifically, values within 10% of the widest existing reference intervals were excluded (n=16; final sample size n=108). Difference plots were also used for comparison and bootstrap resampling was used to calculate confidence intervals.

Results: CSF-TP results were slightly lower on the Siemens Advia compared with the Vista reference method. Passing-Bablock regression yielded a slope of 0.99 (0.96-1.03) and y-intercept of -0.05 (-0.06-0.03; r^2=0.86). Median values on the Advia (0.379 g/L) were 0.071 g/L (~15%) lower than the Vista (0.45 g/L). This difference was within the allowable error criteria defined by the College of American Pathologists. **Conclusions:** The Advia CSF-TP method runs slightly lower than the Vista method. The findings herein are consistent with CAP proficiency testing data. The difference of ~15% is within acceptable total error limits (the greater of 20% or 2 SD). We conclude that the recently determined age and sex partitioned intervals for the Vista, Roche, and VITROS are applicable to the Advia method. Overall this work extends previous studies showing age and sex partitioned CSF-TP reference intervals are superior to a single text-book derived cutpoint.



A-050

Chromatographic Separation of Biomarkers of Mucopolysaccharidoses (MPS) Type IVA/VI using a Focused Gradient UPLC-MS/MS Method

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Background: Morquio A (MPS IVA) and Maroteaux-Lamy syndromes (MPS VI) are rare autosomal recessive genetic disorders caused by deficiency of the two lysosomal enzymes N-acetylgalactosamine 6-sulfatase and N-acetylgalactosamine 4-sulfatase, respectively. This leads to impaired degradation and, consequently, accumulation of the glycosaminoglycans (GAGs) chondroitin (CS)/ keratan sulfate (KS) in MPS IVA and dermatan sulfate (DS) in MPS VI. The two Non-Reducing Ends (NRE) of chondroitin/dermatan sulfate, a6 (GalNAc6S), and a4 (GalNAc4S) are biomarkers specific for MPS IVA and MPS VI respectively. Upon digestion with chondroitinases, CS/DS GAGs will release the NREs a4 and a6. NREs are increased in patients with MPS IVA and VI, and they can be used for diagnosis and therapy monitoring of these patients. They are positional isomers and their identification depends on good chromatographic separation. We have optimized the chromatographic separation of these positional isomers using a focused gradient UPLC-MS/MS method that allows accurate identification and quantitation of the two NREs, a4 and a6. Method: GAGs were isolated by anion exchange chromatography and digested using specific lyases to release the biomarkers. The biomarkers were then differentially mass-labeled by reductive amination with [$^{12}C_6$] aniline. Samples were analyzed using Waters Xevo TQS Triple quadrupole mass spectrometer operated in negative ion SRM-mode and with Acquity UPLC system (Waters). A reverse-phase C18 column (Waters CSH, C_{18}, 1.7µM, 2.1 x100 mm) was used to separate analytes. The flow rate was 350 µl/minutes, and injection-to-injection run time was 27 minutes. The mobile phase A was Water with 8 mM glacial acetic acid and 5 mM dibutylamine (DBA), and mobile phase B contained 70% Methanol/Water with 8 mM glacial acetic acid and 5 mM DBA as additives. The analytes were separated using a focused gradient method, calculated by determining the percentage B at which isomers elutes and calculating how much % B changes per column volume over time in a shallow gradient. Results: The focused gradient method eluted a6 and a4 at 9.95 minutes and 10.34 minutes, respectively. The method was linear (r^2 >0.99) across the analytical measurement range for both analytes. The precision of the back-calculated concentrations of the lowest calibrator (LLOQ, 0.01 mg/L for both analytes) and highest calibrator (ULOQ, 12 mg/L for a6 and 6 mg/L for a4) over 6 independent runs was ≤5.3% for a6 and ≤ 1.6 % for a4. The mean accuracy was ≤ 8.5% with intra-assay precision and inter-assay precision ≤ 4%.

Conclusion:

A sensitive assay to detect MPS IVA/VI specific biomarkers was developed. The focused gradient UPLC-MS/MS method increased the resolution without increasing the run time. This assay would allow accurate diagnosis and targeted therapy monitoring of patients with these disorders.

A-051

Beckman DxC700AU: Technical Evaluation and Upgrade in a Tertiary Restructured Hospital

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Background: Singapore General Hospitals' clinical biochemistry laboratory invests heavily into total laboratory automation and improvements in automation processes to manage the increasing demands of biochemical analysis. Beckman Coulter had released its newest chemistry analyzer; the DxC700AU, which was demonstrated to have improved features to its series predecessor, the AU680. These features include newer, streamlined functions that increases work efficiency and indirectly improving patient safety. The laboratory aimed to evaluate and verify the performance parameters of 19 analytes on Beckman Coulter's newest automated chemistry analyzer in view of upgrading its current AU680.

Methods: The analytes evaluated include serum urea, sodium, potassium, chloride, bicarbonate, glucose, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, alanine transaminase, aspartate transaminase, gamma glutamyl transaminase, calcium, inorganic phosphate, magnesium, creatine kinase and amylase. Parameters verified include linearity, limit of detection (LOD), carryover, and imprecision. Patient comparison was also performed between the current AU680 and DxC700AU analyzer. Linearity was verified within the analytical measuring range (AMR) by performing triplicate measurements using Validate-It linearity materials or by diluting a high concentration patient matrix at 25%, 50% and 75% of the neat specimen. LOD was verified by analyzing 20 replicates of a blank matrix (saline) and 20 replicates of a patient specimen with concentrations near the LOD. Carryover was assessed by analyzing a low concentration sample in triplicates, a high concentration sample in duplicates followed by the same low concentration sample in duplicates consecutively. Imprecision was assessed in triplicates across 5 days using quality control material from Beckman Coulter. Lastly, patient comparison was performed using 60 to 180 samples for each analyte between the DxC700AU and the laboratory's AU680 analyzer. Analyze-It v2.03 was used to generate Passing Bablok Regression, Spearman Correlation and Altman-Bland Bias Plots from the comparison data.

Results: Total imprecision for all the analytes were generally <1.98%. All analytes were verified to be linear within their respective AMRs. LOD was assessed to be satisfactory and below the manufacturer's claimed lower limit of measurement. Linearity for each analyte was assessed and verified across >75% of the AMR with acceptable recoveries ranging from 94% to 107%. No significant carryover was observed for any analyte. Passing Bablok Regressions, where X is the AU680 and Y is the DxC700AU, generated slopes ranging between 0.99X and 1.06X with constants ranging between -2.11 and 1.51. Altman-Bland Bias Plots presented bias ranging between -4.8% and 5.0% for different anaytes. All analytes had excellent correlation between the 2 analyzers with R values ranging from 0.99 to 1.0.

Conclusion: The DxC700AU had demonstrated acceptable parameter performance comparable to the AU680. The excellent comparability and correlation of results eases transference of the assays from the AU680 to DxC700AU. The DxC700AU offers many new features previously lacking on AU680, including an LED indicator that alerts staff to instrument errors and reagent loading on the fly allows staff to easily troubleshoot reagents with issues. These new features of the DxC700AU improve quality of work life, which is particular important in the intense work environment of a 24-hour laboratory offering critical services.

A-052

Abbott Alinity ci System Sigma Metrics for Urine Assays

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Background: Sigma metrics are an efficient way for determining product quality, comparing a combination of two metrics - precision and bias - relative to a total allowable error (TEa) goal. A higher sigma metric corresponds to a higher quality product where 6 sigma is considered a world-class quality performance metric. This study was conducted on urine applications using the Alinity ci system to determine sigma metrics for 1 immunoassay and 11 clinical chemistry assays. The results of this study complement the sigma metrics reported for more than 100 serum/plasma applications on immunoassays and clinical chemistry assays tested on the Alinity ci system and presented in posters at the 2017, 2018, and 2019 AACC meetings. **Methods:** A sigma metric was calculated for each assay using the equation: sigma = (TEa - bias)/precision, where the 2014 allowable limits of performance from the Royal College of Pathologists of Australasia (RCPA) was used as the TEa goal. The bias was estimated at a medically relevant low concentration using the Passing-Bablok linear regression model from a study comparing the first replicate of the Alinity result to the mean of the ARCHITECT system results. The study was conducted per CLSI EP09 by testing a minimum of 40 samples across the analytical measuring interval on the Alinity ci and on the Abbott ARCHITECT *c*8000 or *i*2000 systems. For precision, the within-laboratory standard deviation (SD) or percent coefficient of variation (%CV) was determined near a medically relevant low concentration from a study conducted per CLSI EP05 where controls and panels were tested in replicates of 2 - 3 during 2 runs per day for at least 20 days. **Results:** Nine of the twelve assays (75%) demonstrated at least 6 sigma performance, while two assays demonstrated 4 sigma performance, and one assay demonstrated 2 sigma performance. **Conclusion:** A majority of the Alinity ci assays demonstrated at least 6 sigma performance for the urine application. These results are comparable to the sigma performance observed on the more than 100 previously studied Alinity ci system assays for the serum/plasma application. Sigma metrics can be a useful tool for laboratorians to use to compare and monitor assay performance to ensure high quality healthcare for patients.

A-055

Stable Unconjugated Bilirubin Solution: Potential Usage as Calibrators and Controls

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Objective: To develop a stable high concentration aqueous bilirubin stock solution. **Background:** Bilirubin is the photosensitive degradation product of hemoglobin generated during RBC catabolism. Commercially available unconjugated bilirubin is practically insoluble in water and is soluble in some organic solvents. Bilirubin is also one of the major interferences for hemolysis estimation in blood samples. Chemically-synthesized bilirubin analogues can be used as calibrators with the maximum concentration being 20 mg/dL. Existing bilirubin calibrator products have several drawbacks pertaining to re-constitutional errors (when supplied lyophilized), shorter shelf-life and bilirubin oxidation to biliverdin remains unaddressed. Currently the maximum concentration of an aqueous unconjugated bilirubin is 20 mg/dL. **Methodology:** A novel method for dissolving very high quantities of unconjugated bilirubin eliminates the use of organic solvents but instead involves usage of inorganic salts as buffering agents. The formulated aqueous diluent was prepared by dissolving sodium chloride (1 gm/L), potassium phosphate (0.25 gm/L) and urea (2.5 gm/L) at pH 7.0±0.2. The diluent was then adjusted to pH 11.5±0.02 with sodium carbonate and then unconjugated solid bilirubin added in the ratio 4.5:1. The pH of the resulting solution lowered as the bilirubin dissolved and gradually adjusted to physiological pH. To mimic physiological conditions human serum albumin was added to the solution at 40 mg/mL. The maximum concentration of unconjugated bilirubin in this aqueous solution is ~520 mg/dL. This solution was mixed with equal volumes of human plasma to maintain the physiological pH and stabilize the solution from oxidation. The aqueous solution of unconjugated bilirubin (260 mg/dL) was filter sterilized and stored at low temperatures and diluted further in plasma on as needed basis. **Measuring Range and Linearity:** CLSI guidelines specify the clinically relevant range for total bilirubin as 0-40 mg/dL. Hence, the prepared bilirubin solution was diluted to 40 mg/dL in sterile plasma and quantitated by a commercially available kit. Further serial dilutions behaved linearly ($R^2 \geq 0.99$) when tested spectrophotometrically. **Stability:** Light controlled stability study of the final solution, 40 mg/dL, was conducted over six months and four different storage conditions. At room temperature (15-30°C) and at 2-8°C the solution exhibited significant instability beyond 7 days and 30 days respectively. At -20°C the sample integrity was unaltered across 6 months. At -80°C, a larger standard deviation was noted across months as compared to -20°C. **Precision and Accuracy:** Standard deviations of stable solutions across various days, different temperatures and sample replicates were limited to ±0.05 OD. **Results:** A high concentration aqueous solution of 260 mg/dL and a stable working solution of 40 mg/dL for unconjugated bilirubin was achieved having long shelf-life of at least six months at -20°C with restricted inter-day variability. **Conclusion:** This stable high concentration aqueous solution of unconjugated bilirubin could be used to prepare clinically relevant bilirubin calibrators and controls.

A-057

Performance of the Optilite Alpha-2-macroglobulin Urine Assay for Use on the Binding Site Optilite® Turbidimetric Analyser

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Background: The Optilite® alpha-2-macroglobulin urine (α2M Urine) assay is intended for the quantitative in vitro measurement of α2M in urine using the Binding Site Optilite analyser to aid in the diagnosis of post-renal haematuria. α2M is a high molecular weight (725 kDa) glycoprotein that has a broad-spectrum serine protease inhibitor function. The presence of α2M in the urine is significant in the differentiation of glomerular and post-renal haematuria, in particular when albumin levels are greater than 100 mg/L as the glycoprotein would normally be filtered by the glomerulus leading to its absence in urine and almost exclusive distribution in the intravascular pool. Increased α2M in the urine may be a sign of post-renal haematuria. α2M is also involved in the transport of hormones, cytokines and metals such as zinc. Here we describe the evaluation of the alpha-2-macroglobulin urine assay (manufactured by The Binding Site Ltd, UK) for the Binding Site Optilite analyser.

Methods: The measuring range of the assay is 2.7 - 85.0 mg/L at the standard 1+0 dilution established based on CLSI guideline EP28-A3c. A precision study was performed according to CLSI guideline EP15-A3 over a period of 6 days using one reagent lot on one analyser. The study was carried out using 3 urine samples with different analyte concentrations including within 75-90% of the standard measuring range top point. Six replicates were run against six calibration events. A comparison study against the Siemens BN™ II System assay was performed using 170 samples ranging from 2.7 - 49.3 mg/L based on CLSI guideline EP09-A3. A Limit of Quantitation (LoQ) verification study was based on CLSI guideline EP17-A2. A linearity study was performed following EP06-A. Interference testing was performed following CLSI guideline EP07-A2 using 13 potential drug and metabolite interferents including calcium chloride, gamma globulin and sodium oxalate at 3 testing levels.

Results: The total precision coefficients of variation (CVs) were as follows: 8.3% at 5.26mg/L, 4.9% at 13.65 mg/L and 3.6% at 76.65 mg/L. Within run precision resulted in acceptable %CV of <7.4% and between lot/instrument CV of <2.3%. Weighted Deming fit analysis of comparison data for α2M Urine comparing the Optilite (test assay) and BNII (predicate assay) showed good agreement with an equation of y=0.9235 x + 0.4394. The assay has antigen excess protection up to 100 mg/L. The assay has an LoQ claim of 2.7 mg/L with the highest %CV of 5.90%. The assay gave a linear response over the analyte range of 2.7 - 85.0 mg/L with deviation from linearity <10%. Interference Testing for α2M Urine Optilite yielded no significant (>10%) interference with all tested materials.

Conclusion: The Alpha-2-Macroglobulin urine assay for the Optilite provides a reliable and precise method for quantifying Alpha-2-Macroglobulin content in human urine and correlates well with existing methods.

A-060

Foam Padding in Pneumatic Tube Carriers Reduces G-Forces and Sample Hemolysis Rates

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Introduction: Hemolysis is a major cause of blood-specimen rejection, with especially high rates in the neonatal intensive care unit (NICU) and emergency department (ED). Previous studies have reported increased hemolysis rates when transporting specimens by pneumatic tube system (PTS), the extent of which can be correlated with increasing g-forces experienced by the sample. Here we evaluated the hemolysis rates and g-forces before and after the implementation of foam-padded PTS carriers as part of an effort to improve specimen quality from high-rejection rate patient care areas. **Methods:** Hemolysis rates were evaluated for specimens collected in the NICU and transported non-centrifuged by PTS using an unpadded carrier or a foam-padded carrier. Both were then compared to baseline (centrifugation in the NICU and walking

samples to the lab). Hemolysis was determined by a combination of visual inspection and/or LIS comments/rejection based on hemolysis index (Roche Diagnostics). In a follow-up experiment, the g-forces a sample would experience during PTS transport was measured using a VitalVial (Motryx, Inc.) for the NICU and ED. The cumulative g-forces were represented by the 'VitalMetric', calculated by detecting peaks in the acceleration vector sum of the x, y and z components, weighting the peaks by half of their magnitude, and calculating the right-tailed area under the weighted vector sum acceleration distribution above 2 g. The VitalMetric therefore represents the combined 3-axis effect of accelerations over a transit's duration. **Results:** The NICU visual hemolysis rate using unpadded PTS carriers was 61% (92/150), of which 11% (17/150) resulted in sample rejection due to severe hemolysis. At baseline, centrifuging in the NICU prior to walking samples to the lab demonstrated a hemolysis rate of 27% (16/59) with no sample rejections due to hemolysis. Embedding the non-centrifuged specimen in foam padding within the PTS carrier produced a visual hemolysis rate equal to unpadded carriers at 61% (32/56), however eliminated sample rejections due to hemolysis (0/56). PTS transport from the NICU using unpadded carriers had a mean(±SD) VitalMetric of 970.7±44.4, which was reduced 30% to 746.4±24.1 in padded carriers. VitalMetric values were higher when transporting from the ED (1447.9±69.2; a longer PTS route) but were reduced 26% when foam padding was used. **Conclusions:** Using foam-padded carriers for PTS transport decreased g-forces experienced and eliminated sample rejections due to hemolysis in the NICU. A similar decrease was observed for foam-padded carriers from ED. The VitalMetric is a useful indicator of g-forces experienced by blood tubes, correlates with hemolysis and can be used as a tool to assess the efficacy of PTS transport for blood samples.

A-061

WHO 07/202 and Two Serum-based Materials are Commutable for Soluble Transferrin Receptor

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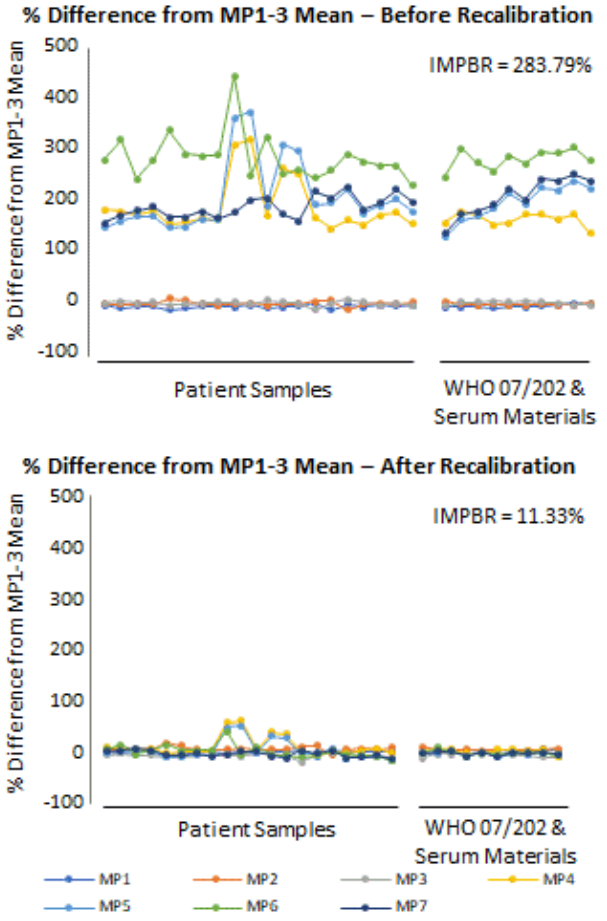
Background: Iron deficiency is the leading cause of anemia and affects >2 billion people. A 2004 Joint WHO/CDC Technical Consultation recommended the measurement of serum ferritin and soluble transferrin receptor (sTfR) to assess iron deficiency anemia in populations. However, the measurement of sTfR presents challenges due to a lack of assay standardization. In 2009, the WHO/NIBSC released a recombinant sTfR reference material called 07/202. This study evaluated the commutability of WHO 07/202 and C37 and non-C37 compliant serum materials from 2 independent manufacturers using 6 different measurement procedures (MPs). **Methods:** The study included 20 individual donor serum samples that spanned the healthy sTfR range (1.65-5.40 mg/L) and that exceeded this range, indicating iron deficiency. Results were analyzed using the 2018 IFCC Working Group's "Recommendations for Assessing Commutability". Materials were considered commutable if they behaved like patient samples. The impact of recalibration to these reference materials was estimated by evaluating the inter-measurement procedure bias range (IMPBR) across the MPs before and after recalibration. **Results:** Due to the lack of a reference measurement procedure and the non-normal distribution of data from different MPs, the mean of MPs1-3 was used as the target instead of the trimmed mean of all MPs. MPs1-3 exhibited no patient sample interferences and median biases and standard deviations of ≤4.6% before recalibration. The WHO 07/202 material appears commutable based on overlapping 95% confidence intervals. A second harmonization study is underway to fully assess the commutability of 07/202. Both serum pools were commutable. The IMPBR across all MPs was 283.79% before recalibration. Recalibration to the C37 or the non-C37 serum pools equally and significantly reduced the IMPBR to 11.3% and 9.5%, respectively. **Conclusion:** WHO 07/202 and 2 serum-based materials are commutable. Recalibration using these materials significantly reduced the IMPBR across MPs and improved sTfR measurement agreement in patient samples.



A-062

Performance Evaluation of the Atellica CH Enzymatic Hemoglobin A1c Assay on the Atellica CH Analyzer and Comparison with the Sebia CAPILLARYS 2 FLEX PIERCING Instrument

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Background: A hallmark of uncontrolled diabetes is hyperglycemia which, if left unchecked, can lead to severe organ system damage. Early diagnosis is key for disease management, preventing progression, and identifying those at risk of developing diabetes. Glycated hemoglobin (HbA1c) is a glycemic marker that reflects long-term (approximately 8-12 week) plasma glucose concentrations. HbA1c assays are used to aid in the early diagnosis of diabetes, diabetes management, and identifying those at increased risk of developing disease. The new Siemens Healthineers Atellica® CH Enzymatic Hemoglobin A1c (A1c_E) assay specifically measures N-terminal fructosyl dipeptides on the beta-chain of HbA1c. The assay is NGSP-certified and data in the manufacturer's package insert show good correlation compared to HPLC and <5% change with most A1c variants; it measures mmol/mol HbA1c (IFCC) and %HbA1c (DCCT/NGSP) in whole blood and hemolysate on the Atellica® CH Analyzer. The goal of this study was to verify NGSP and IFCC performance specifications for the Atellica CH A1c_E assay imprecision and compare assay performance with the Sebia CAPILLARYS 2 FLEX PIERCING Instrument in our laboratory.

Methods: Imprecision was evaluated according to CLSI Document EP15-A2. Two levels of commercially available Lyphochek® Diabetes HbA1c controls (4.59% and 9.01%) were tested. Each sample was assayed in triplicate, one run per day, for five days (n=15 per level). A method comparison study was performed between the Atellica CH A1c_E Assay and the CAPILLARYS 2 FLEX PIERCING Instrument according to CLSI Document EP09-A2. Each run was made after daily quality control was performed.

Results: Repeatability ranged from 0.4%CV to 1.0%CV. Within-laboratory imprecision ranged from 0.4%CV to 1.0%CV. Both met CLIA requirements. The method comparison study yielded the following linear regression equation: Atellica CH A1c_E = 0.962[CAPILLARYS 2 FLEX PIERCING Instrument] - 0.049% HbA1c; R^2=0.9956 (n=40); slope=0.962 (95% confidence interval, CI 0.941 to 0.983); Intercept=0.049 (95% CI -0.202 to 0.104). Deming (y=0.964x-0.063) and Passing Bablok (y=0.931x+0.143) regression provided similar results. These results verify the manufacturer's claims and alignment to NGSP requirements.

Conclusion: The Atellica CH A1c_E assay meets the precision requirements defined by CLIA; therefore, fulfills the intended use requirements. The method comparison results obtained for assays on the Atellica CH Analyzer and CAPILLARYS 2 FLEX PIERCING Instrument are comparable; therefore, the Atellica CH A1c_E assay is fit for use in a clinical laboratory setting.

A-063

Performance Evaluation of Representative Clinical Chemistry Assays for C-reactive Protein (CRP) on the Alinity c Analyzer from Abbott Laboratories

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Background: The CRP Wide Range (WR), High Sensitive (HS), and Cardiac assays are intended for the quantitation of CRP in human serum and plasma with variable assay ranges. CRP Cardiac assay is to be used as an aid in identification and stratification of individuals at risk for cardiovascular disease.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 the CRP WR, HS, and Cardiac applications on the Alinity c analyzer, utilizing photometric technology for the quantitative determination of CRP 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 reportable measuring interval, analytical measuring interval and extended measuring intervals were determined based on guidance from CLSI EP34. **Results:** The observed results for precision, LoQ, method comparison, and defined analytical and reportable measuring intervals for CRP assays are shown in the table below.

	CRP HS	CRP WR	CRP Cardiac
TotalSD/%CV	≤ 0.005 SD ≤ 2.6 %CV	≤ 0.012 SD ≤ 1.9 %CV	≤ 0.033 SD ≤ 2.8 %CV
LoQ	0.03 mg/dL(0.30 mg/L)	0.10 mg/dL(1.0 mg/L)	0.30 mg/L
Method Comparison to ARCHITECT (Slope / Correlation)	0.98 / 1.00	1.01 / 1.00	0.97 / 1.00
Analytical Measuring Interval (AMI)	0.03 to 16.00 mg/dL(0.30 to 160.00 mg/L)	0.10 to 48.00 mg/dL(1.0 to 480.0 mg/L)	0.3 to 10.00 mg/L
Extended Measuring Interval (EMI)	16.00 – 1600.00 mg/dL(160.00 – 16000.00 mg/L)	48.00 – 240.00 mg/dL(480.00 – 2400.00 mg/L)	N/A
Reportable Interval (RI)	0.02 to 160.00 mg/dL(0.20 to 1600.00 mg/L)	0.04 to 240.00 mg/dL(0.4 to 2400.0 mg/L)	0.10 to 10.00 mg/L

Conclusion: Various applications of the CRP assay utilizing photometric technology on the Alinity c analyzer demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT CRP assay.

A-064

Management of Vitamin B12 in the Biochemistry Laboratory

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Background: Vitamin B12 is a water-soluble vitamin, important in protein metabolism, in the formation of red blood cells and in the maintenance of the central nervous system. This vitamin is found naturally in animal foods, such as fish, meat, eggs and dairy products.Vitamin B12 deficiency affects the synthesis of erythrocytes, causing megaloblastic anemia by abnormal DNA synthesis, which leads to an increase in the mean corpuscular volume (MCV) of red blood cells. Early diagnosis is very important since its deficit for more than 6 months can cause irreversible cognitive impairment. The objective of this study was to analyze the requests with a joint application of vitamin B12 and MCV, and implement a protocol with adequate demand for vitamin B12 for a better use of the resources present in the Biochemistry Laboratory.

Methods: Descriptive and retrospective study of all requests for vitamin B12 received in the laboratory during 2019. The determination of vitamin B12 was performed on a Cobas 8000 analyzer (Roche Diagnostics) by immunoassay, while the MCV was obtained from the blood count, measured by conventional techniques. The population older than 60 years was excluded from the study due to a high prevalence of vitamin B12 deficiency.

Results: During the study period, 51,072 requests for blood count and vitamin B12 were analyzed. Of these, 45,607 (89.3%) had a MCV within normal or decreased, and in only 5,465 (10.7%) of the applications the levels of MCV were high. Among the cases with normal or decreased MCV, in 760 applications (6%), there was a deficit of vitamin B12.

Conclusion: It would be necessary to implement a demand adequacy protocol to avoid unnecessary tests and make rational use of laboratory resources.Because of this, the determination of vitamin B12 should not be performed if there is a normal or low value of the MCV, except in cases where there is clear clinical suspicion or in a population over 60 years. Although with the proposed protocol the determination of vitamin B12 had been avoided in 89.3% of the cases, we consider that it is necessary to find a balance between cost savings and carrying out the test to reduce cases of undiagnosed vitamin B12 deficiency.

A-068

Amino Acid Profile of Ornithine Transcarbamylase Deficiency from a Fibrolamellar Hepatocellular Carcinoma Patient Compared with the Congenital Case

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Background: Ornithine transcarbamylase deficiency (OTCD) is the most common urea cycle disorder caused by pathogenic variants of *OTC* gene on X chromosome. Most of OTCD patients are male and typically show hyperammonemic symptoms within three days of life despite being healthy at birth. However late-onset OTCD arises from previous heathy adults and some of them have no pathogenic variant in *OTC* gene. In cases of fibrolamellar hepatocellular carcinoma (FL-HCC), a rare variant of hepatocellular carcinoma which is frequent in adolescent and young adult, few patients were diagnosed as OTCD during treatment. The patients were rarely reported in East Asia. To make an exact diagnosis, urine organic acids and plasma amino acids were measured in these patients. Here we report the FL-HCC OTCD patient in Korea and compare lab findings with the congenital OTCD pateint.

Methods: Two patients visited emergency medical center of Seoul national university hospital. The patient A was a 3-day-old boy. He was healthy at birth, but was in semi-coma and hypothermia status at arrival. And the patient B was an 18-year-old male who was diagnosed as FL-HCC stage IV. He was admitted for mental change with psychotic behavior after three days of his first chemotherapy. In the initial lab findings, they showed high level of serum ammonia, but did not have signs of metabolic acidosis. Both patients were tested for urine organic acids and plasma amino acids at the initial work up. Molecular test for both patients to check variants of *OTC* gene was done.

Results: In patient A who had congenital OTCD, peak ammonia level was 2014 µmol/L and in patient B who had FL-HCC, peak ammonia level was 454 µmol/L. Hyperammonemia of both patients were resolved after 4 to 6 days of continuous renal replacement therapy. The urine orotic acids were elevated to 296.3 mmol/mol Cr in

patient A and 226.9 mmol/mol Cr in patient B. Citrulline was extremely decreased to <1 µmol/L in patient A, and 8 µmol/L in patient B. These patterns were maintained at the follow-up exam in one month respectively. Glutamine and alanine were elevated in both patients with hyperammonemia. After hyperammonemic event, molecular tests revealed that there was a pathogenic variant only in patient A (*OTC*, c.274C>T, p.Arg92*, heterozygote).

Conclusion: The FL-HCC patient had no pathogenic variant of *OTC* gene. Although hyperammonemia was less severe in the FL-HCC patient, urine orotic acid was elevated to similar level of the congenital OTCD patient. Plasma amino acid profile showed that glutamine and alanine were elevated and citrulline was markedly decreased in both patients.

A-069

Reference Measurement Procedure for the Determination of Glucose in Serum by Isotope Dilution Gas Chromatography-Mass Spectrometry

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Background: Laboratory measurements are critical for the correct diagnosis and treatment of patients as well as in the investigation of diseases, such as diabetes. Inaccurate and unreliable measurements can lead to the misclassification of patients and incorrect treatment. According to the American Diabetes Association, 86 million people in the USA have diabetes. Glucose is one of the main biomarkers in diabetes management and is measured in both laboratory and at-home settings (with the use of point of care devices). With various studies reporting inaccurate measurements, especially in pathological glucose concentration ranges, there is a public healthcare need to improve accuracy and precision of glucose measurements. An isotope dilution-gas chromatography-mass spectrometry (ID-GC-MS) RMP for glucose in serum has been developed and validated at CDC and is considered the only currently operating reference method for glucose in the USA.

Methods: The principle of the CDC glucose RMP is based on previously published methods. Samples were weighed on an analytical balance and spiked gravimetrically with labeled internal standard (IS) (D-glucose- $^{13}C_6$). The samples underwent protein precipitation with methanol, followed by two derivatization steps with hydroxylamine hydrochloride in pyridine and acetic anhydride, respectively to form the aldononitrile acetate. The samples were then diluted in ethyl acetate before analysis on the GCMS. Analysis was performed using a 6890/5975 Agilent GCMS system equipped with a Zebron ZB-50 capillary column of 30 m length x 0.25 mm internal diameter x 0.25 µm film thickness (Phenomenex). The selected ions for quantitation were 314 and 319 m/z that corresponded to fragmented ions from glucose and IS. A six-point calibration curve ranging from 13.50 mg/dL (0.75 mmol) to 378.00 mg/dL (21 mmol) was used for each assay with each calibrator analyzed in duplicate. NIST SRM 917c, D-Glucose (Dextrose) was the primary reference material to ensure traceability to SI. Four levels of serum-based reference material NIST SRM 965b and four levels of Laboratoire National De Metrologie Et D'essais (LNE) CRMs were analyzed in four replicates over five days to evaluate accuracy of the method. The measurement results were compared to the certified values and NIST values were evaluated for agreement using NIST Special Publication 829.

Results: Method validation results showed great reproducibility with a within-run precision of 0.85%, 0.60% and 0.42% and among run precision of 0.52%, 0.53% and 0.66% for 3 levels of low, medium and high quality control materials, respectively. The method showed excellent accuracy with a bias of 0.77%, 0.84%, -0.14% and -0.70% for four levels of NIST certified reference material and 0.30%, -0.79%, -0.19%, -0.57% for four levels of LNE CRM. The calibration curve was linear from 13.50 mg/dL to 378.00 mg/dL (R2 =0.9999).

Conclusion: An accurate and reproducible method was successfully developed for measuring glucose in serum samples. The method is currently used to assign values for accuracy-based proficiency testing ABGIC CAP program and in the future will allow for accuracy evaluation of comparator devices such as YSI. This RMP will be used to assign target values to serum materials used to establish standardization programs for glucose.

A-070

Detection of Diethylene Glycol Metabolite in Patients with Suspect of Intoxication after Beer Contaminated Consumption in Brazil

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Background: Diethylene glycol is a chemical substance used in the production of a wide variety of commercial and industrial products. It is found in trace, non-harmful amounts in other products such as some dietary supplements and cosmetics. When ingested in large amounts (>1-1.5 g/kg), diethylene glycol can be a potent nephrotoxic and neurological poison. Unfortunately, it present similar physical and chemical properties to solvents such as ethylene glycol, propylene glycol and glycerin used in beverage cooling or pharmaceuticals formulations and intoxication cases was reported. Diglycolic acid is a metabolite of diethylene glycol and was found in elevated concentrations in serum samples of 2006 Panama intoxication cases, and prove to be more specific than other metabolites as 2-hydroxyethoxyacetic acid (HEAA). In this work, we evaluate the presence of diglycolic acid in serum or plasma samples of 9 suspected intoxication cases and 21 negative controls. **Methods:** The analysis was performed in GC-MS after a protein precipitation, liquid-liquid extraction and BSTFA derivatization. In total, 29 samples from 9 intoxication suspected cases and 21 samples from 21 donors used as control were analyzed. **Results:** There was no detected the presence of diglycolic acid in any control samples, and the metabolite was detected in 8/9 suspected cases in at least in one sample. In one suspect case, no one of the five samples collected in four different days were detected. In this case, the physicians noticed a different clinical evolution from the others suspected cases. In other patient treated with hemodialysis, the first sample collected after the procedure (<12h) was negative for diglycolic acid and the later samples were positive again. **Conclusion:** In conclusion, the diglycolic acid appear to be a useful biomarkers for detect the human diethylene glycol intoxication. The metabolite quantification may be useful to helpful the physicians during the treatment with ethanol and hemodialysis. Despite the good sensibility of the metabolite detection for intoxication by diethylene glycol, further studies are necessary to elucidate how the measurement can helpful the physicians during intoxication treatment.

A-072

Urine Biochemistry External Quality Assessment: Evaluation of Performance through Results of the First Distribution in Buenos Aires EQAS.

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Background: ProgBA-CEMIC ("Buenos Aires" External Quality Assurance Program for Clinical Laboratories, accredited under ISO/IEC 17043) has been introducing new specialties since 1979. At present, matrixes distributed are: serum/plasma, whole blood and paper with 142 analytes assessed. We present performance results of 24 hours urine biochemistry specialty (14 analytes), introduced and accredited in 2019. **Methods:** Four freeze-dried urine pools were distributed in 12 samples, assessed monthly, to 101 laboratories from Argentina and other Latin American countries. Major platforms used by participants were: Roche Cobas, Abbott Architect, Siemens Dimension, Ortho Vitros. Pool target values were assigned as peer group consensus means. Acceptance limits were set as ±3SD. CV% between laboratories for each pool and analyte were computed. All Labs CV% were calculated as weighted mean of each pool CV%. Each laboratory mean CV% (CVi%) was calculated through individual results of same pool samples repeated throughout the year. Mean CVi% for each 14 analytes was compared with established quality requirements (QR): Biological Variability, Czech EQA or Spanish EQAP organizers.

Results: Samples showed very good stability, pool average mean CV <0.8% along the year, except for very low concentration of some analytes. The percentage of laboratories achieving target CVi% < QR for each parameter, surpassed 71% (highest 92% for U-Na). Exceptions: Urinary Free Cortisol (UFC) < 44%, due to low number of participants and lack of methods´ standardization (immunoturbidimetric, nephelometric, chemiluminiscent); U-Glucose < 44%, U-inorganic Phosphate < 51% and U-Total Protein < 66%, due to some pools´ low analyte concentration. **Conclusion:** First ProgBA distribution for urine biochemistry showed an acceptable percentage of laboratories achieving the proposed QR. External Quality Control is a useful tool for monitoring methods´ performance. ProgBA EQAS results reflect the state of the art of IVDs in our region.

Analyte	N	All Labs CV%	Mean individual Lab CVi%	Quality Requirement % (QR)	% Labs with CVi% < QR
U-Uric Acid	76	8.1	8,4	19.4 (BVi)	88
U-Ca	76	9.3	10	34.1 (BVi)	88
UFC	18	47	36	30 (*)	44
U-Cl	69	18	7,9	14 (DMAX SEKK)	71
U-Creatinin	79	6.5	8,0	15.4 (BVi)	72
U- Glucose	55	42	26	22 (DMAX SEKK)	44
U-K	77	5	6,8	28.4 (BVi)	91
U- Albumin	65	24	25	40.6 (BVi)	82
U-Mg	67	17	21	45 (BVi)	82
U-Na	77	8.5	7,8	32 (BVi)	92
U- Osmolality	13	25	6,7	-	-
U-Protein	73	27	23	34 (EQAP)	66
U-P	74	8.3	26	28.4 (BVi)	51
U-Urea	77	6.9	8,4	22.1 (BVi)	73

BVi = Biological Variability; (*) Clinica Chimica Acta 309; 25–35. 2001; SEKK=Czech EQA program. EQAP= Spanish EQAP organizers (2015).

A-073

Solid Phase Extraction of Plasma Specimens for Arginine Vasopressin Hormone Measurement by Polymeric Reversed-Phase Sorbent Columns

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Background: Arginine vasopressin hormone (AVH) is a nine amino-acid peptide which is cyclic in nature. It functions as an antidiuretic by acting on the collecting tubules of the kidney, causing an increase in permeability to water and urea but not to larger molecules. AVH also possesses neurotransmitter functions, as well as releasing factor and peripheral humoral functions. AVH determination is useful in the study of diabetes insipidus, psychogenic water intoxication, hyponatremia, stress conditions, and hypertension studies. AVH measurement is challenged due to its small size, low concentrations, and manual assay techniques. We aimed to improve our current method of AVH measurement by evaluating an updated protocol for extraction of AVH from human plasma samples.

Methods: The EURO-DIAGNOSTICA AVH RIA (IBL America, Minneapolis, MN) consists of two steps. The first involves solid phase extraction (SPE) of plasma specimens, then the extracted specimens are assayed using a disequilibrium RIA procedure to maximize sensitivity. Our current SPE step uses octadecasilyl-silica (Waters Sep-Pak C_{18}) cartridges attached to syringes on Vac Elut vacuum manifolds. This method is manual, time-consuming, and requires ample reagent volume. We evaluated an extraction method that used polymeric reversed-phase sorbent columns (Waters Oasis HLB, Milford, MA) and positive pressure workstations. A 3-step SPE protocol was developed to consist of load, wash, and elution steps (eliminating conditioning and equilibration steps). Imprecision studies used manufacturer's controls (26.7 and 8.6 pg/mL) and patient pool (14.5 pg/mL) assayed in triplicate, once daily for 5 days. Limit of detection (LOD) was assessed by measuring the 0.0 and 2.0 pg/mL calibrators 12 times each. Linearity was determined by serially diluting a high AVH sample with a low AVH sample to create 5 samples tested in duplicate. 30 samples, spanning the analytical measurement range, were measured using both SPE protocols.

Results: Between-day CVs were 15.4% at 8.6 pg/mL, 6.2% at 14.5 pg/mL and 8.7% at 26.7 pg/mL, respectively. LOD was 0.5 pg/mL. The assay was linear up to 45 pg/mL. Method comparison by Deming regression yielded y = 0.96 x - 1.04, R = 0.98. The reagent usage was reduced by 86% (from 49 mL to 7 mL per extraction).

Conclusion: The simplified AVH 3-step SPE protocol using polymeric columns with positive pressure workstations increased efficiency and reduced reagent usage. This new method demonstrated favorable correlation with the current method and provided benefits to clinical workflow.

A-074

Analytical Performance Assessment of a Newly Formulated REACH Compliant Amylase Assay for Abbott's Alinity c and ARCHITECT c Systems.

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Introduction: α-Amylase is an enzyme that cleaves carbohydrates and is primarily found in the exocrine pancreas and salivary glands. Amylase testing is primarily used in the diagnosis of acute pancreatitis, which is inflammation due to enzymatic necrosis. The most common causes of acute pancreatitis are gallstones and heavy alcohol consumption. Elevated serum and urine amylase results are observed in patients with acute pancreatitis. A Research Use Only (RUO) Alinity c and ARCHITECT c Systems Amylase assay has been developed for the quantitative determination of α-amylase in human serum and urine.

Methods: The newly formulated Amylase assay is a two-reagent photometric assay for the quantitative determination of α-amylase in human serum and urine. The Amylase assay is a two-part reaction, which improves pre-analytics, in which the substrate ethylidene-4-nitrophenyl-α-(1,4)-D-maltoheptaoside (EPS) is hydrolyzed by α-amylase to form 4,6-ethylidene-α-(1,4)-D-glycopyranosyl-Gx and 4-nitrophenyl-α-(1,4)-glycopyranosyl-G(7-x). The 4-nitrophenyl-α-(1,4)-glycopyranosyl-G(7-x) is then hydrolyzed into glucose monomers and the assay chromophore (4-nitrophenol) by α-glucosidase. The resulting change in absorbance at 404 nm is proportional to the α-amylase concentration in the sample. The assay is traceable to IRMM/IFCC-456 standard with an option to run as calibrated or with a calibration factor.

Results: Total within laboratory imprecision was < 2.2 %CV for the serum application and <1.3 % CV for the urine application. Amylase assay demonstrates linearity up to 3300 U/L for both serum and urine. For the serum application, the limit of quantitation was 2 U/L for ARCHITECT c8 and 3 U/L for Alinity c systems. For the urine application, the limit of quantitation was 3 U/L for ARCHITECT c8 and 2 U/L for Alinity c systems. Reagent onboard stability of at least 30 days was observed for both ARCHITECT and Alinity c systems. The Amylase assay meets CLSI EP 07, 3rd ed. criteria for endogenous interferent levels at amylase concentrations of 50 U/L and 200 U/L, specifically this assay showed robustness to hemoglobin interference (≤ 1000 mg/dL). Method comparison to Abbott's current on-market amylase assay showed a slope of 0.98 with correlation coefficient of 1.00 for the serum concentration range of 6 – 3457 U/L. For the urine application, the slope for the concentration range of 4 – 3203 U/L was 0.96 with a correlation coefficient of 1.00.

Conclusions: A REACH compliant RUO Amylase assay was designed for use with Abbott's ARCHITECT and Alinity c Systems with features including IFCC traceable with the option for a calibration factor or calibrator and is analytically robust.

A-076

Assessment of the Performance of Clinical Chemistry Assays on the Abbott Alinity and ARCHITECT Platforms using the New CLIA Proposed Rules for Acceptance Limits for Proficiency Testing

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Background: In February 2019, new CLIA proposed rules were published in the Federal Register to define new criteria for acceptable performance for proficiency testing. These changes were initiated in 2008 by the Clinical Laboratory Improvement Advisory Committee's recommendation that the acceptance limits for proficiency testing from the original 1992 publication of CLIA rules should be updated to reflect improvements in technology and changes in the utilization of laboratory tests. The new proposed CLIA acceptance limits are more stringent compared to the current acceptance limits. The goal of this study was to assess the performance of the clinical chemistry assays on the Abbott Alinity and ARCHITECT platforms using the more stringent new CLIA proposed rules for acceptance limits for proficiency testing using sigma metrics. **Methods:** The sigma metrics were calculated for 40 different clinical chemistry assays using the current CLIA acceptance limits and then compared to the sigma metrics calculated using the more stringent new proposed CLIA acceptance limits. Sigma metrics data were compiled from a total of 176 assays run on five Alinity instruments and two ARCHITECT instruments. **Results:** For the Alinity platform, using the current CLIA acceptance limits, the percentage of assays that were over 5 sigma, between 3 and 5 sigma and below 3 sigma were 90%, 9% and 1%, respectively. When the new proposed CLIA acceptance limits were used, the percentage of assays that were over 5 sigma, between 4 and 5 sigma, between 3 and 4 sigma and below 3 sigma were 85%, 11% and 4%, respectively. For the ARCHITECT platform, the per-

centage of assays that were over 5 sigma, between 3 and 5 sigma and below 3 sigma were 89%, 11% and 0%, respectively, using the current CLIA acceptance limits. When the new proposed CLIA acceptance limits was used, the percentage of assays that were over 5 sigma, between 4 and 5 sigma, between 3 and 4 sigma and below 3 sigma were 86%, 11% and 3%, respectively. **Conclusion:** The data shows that 85-86% of the assays on both the Abbott Alinity and ARCHITECT platforms had over 5 sigma performance even when the more stringent new proposed CLIA acceptance limits were used to assess the performance of the assays using sigma metrics.

A-077

Analytical and Clinical Performance of the Advansure i3 Procalcitonin Assay

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Background: Sepsis is one of the top 10 causes of death in the world. However, the symptoms of sepsis are not specific, so it is difficult to obtain an early diagnosis. Procalcitonin is clinically useful for early diagnosis and subsequent management of sepsis patients, especially for guiding antibiotic therapy. In general, the concentration of serum procalcitonin in healthy people is lower than 0.1 ng/mL, but in patients with severe bacterial infections and sepsis, the concentration of procalcitonin increases to higher than 2 ng/mL. Serum procalcitonin levels in patients without sepsis can increase significantly under certain circumstances such as after surgery, cardiogenic shock, autoimmune disorders and other conditions. We evaluated the analytical performance of a new automated chemiluminescent immunoanalyzer-based procalcitonin assay, AdvanSure i3 PCT assay on AdvanSure i3 (LG Life Sciences, Seoul, Korea) and compared it with the Elecsys BRAHMS PCT assay on Cobas e801 (Roche, Basel, Switzerland).

Methods: Analytical performance was performed for the precision, linearity, and method comparison by the guidelines of the Clinical Laboratory Standards Institute (CLSI). A total of 119 patient samples were used for comparison in AdvanSure i3 PCT assay and Elecsys BRAHMS PCT assay. Procalcitonin concentrations were <0.25 μg/L in 47 samples, ≥0.25 μg/L and <0.50 μg/L in 21 samples, ≥0.50 μg/L and <2.0 μg/L in 22 samples, and ≥2.0 μg/L in 29 samples. The most widely used medical decision points considered for use of antibiotics are 0.25 ng/mL, 0.5 ng/mL, and 2.0 ng/mL, where the two assays were compared. Clinical evaluation was conducted in 39 patients who tested procalcitonin as an initial assessment of suspected infection. The patients were classified based on the third international consensus definitions for sepsis and septic shock (Sepsis-3). Statistical analyses were performed using LaboStats software.

Results: AdvanSure i3 PCT assay showed good precision with < 5.5% CV for within-run precision and < 6.5% CV for total precision. The assay was linear across the measurement range (0.61-41.08 ng/mL, $R^2 = 0.9992$). Statistical analysis showed that the two assays have good correlation ($r > 0.997$), slope of 1.000 (95% CI, 0.954 to 1.072), and intercept of -0.015 (95% CI, -0.086 to 0.02). The predicted values and 95% CIs for the AdvanSure i3 assay were 0.235 (0.176 - 0.265) ng/mL, 0.485 (0.432 - 0.514) ng/mL, and 1.985 (1.885 - 2.086) ng/mL at three medical decision points, respectively, as calculated by Passing-Bablok regression analysis. The procalcitonin values of the patients with SOFA score <2 (n = 26) or sepsis/septic shock (n = 13) were evaluated. In results with a cut-off at 2.0 ng/mL comparing SOFA <2 with sepsis/septic shock, the sensitivity was 62.5%, the specificity 87.0%, the positive predictive value 76.9% and the negative predictive value 76.9%.

Conclusions: The AdvanSure i3 PCT assay shows good analytical performance and correlation with the Elecsys BRAHMS PCT assay. This new assay can be used for the clinically diagnostic early marker of sepsis in clinical laboratories.

A-078

Photoactivatable Biomimetic Probe as a Potential Tool for Profiling Lectin Specificities

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Background: Many biological processes depend on carbohydrate recognitions to relay critical information and trigger crucial signaling events. Therefore, glycoscience research specifically concerning the carbohydrate-protein interactions can provide an abundant opportunity to discover molecular mechanisms of biological processes, potential therapeutic targets, and diagnostic mechanisms for various diseases. For example, the use of lectins for screening of potential biomarkers has gained increased traction in cancer research, given the development in glycobiology that highlights altered structural changes of glycans on the proteins in cancer associated processes. Lectins, carbohydrate binding proteins that have the properties of recognizing specific carbohydrate moieties of glycoconjugates, can be an effective medium for detection of new biomarkers in complex bodily fluids and tissues. Developing useful tools to comprehensively elucidate the specificity of lectins can provide an added advantage of being the initial key step for selecting specific carbohydrate-lectin interactions for further method development, thus eventually allowing identification of proteins that are differently glycosylated and aberrantly expressed in patients. **Methods:** To achieve the goal of identifying the specificity of lectins for particular glycan structures, a novel lectin probing tool involving affinity photo-crosslinking was designed and its function was demonstrated. The aryl azide chain-end functionalized *N*-glycan polymer was first synthesized from free glycan *via* glycosylamine intermediate followed by acrylation and polymerization *via* cyanoxyl-mediated free radical polymerization (CMFRP) in one-pot fashion. Affinity-assisted photo-labeling capability of the aryl azide *N*-glycan polymers was demonstrated using beta-galactose-specific lectin from *Arachis hypogaea* (PNA) followed UV irradiation and confirmed by SDS-PAGE with silver staining. **Results:** Overall, out of the three synthesized aryl azide chain-end functionalized polymeric probes expressing either glucose, galactose, or lactose, only the probes expressing lactose exhibited PNA specificity, which was consistent with expectations as they processed the beta-galactose moieties specifically known for PNA interactions. The results demonstrated our novel probes' specific capability for binding and labeling lectins based on the lectin's affinity for particular glycol groups on the glycopolymer, whose synthesis can be tailored to test the specificity of a lectin for different glycans. **Conclusion:** The aryl azide chain-end functionalized glycopolymers will be useful and effective biomimetic probes for specific lectin labeling, functionality study and biomarker identification application both *in vitro* and *in vivo*. A wide variety of different carbohydrate moieties can easily be expressed on the novel aryl azide chain-end functionalized polymeric probes to fish for specific lectins of interest, followed by UV irradiation to achieve secure covalent crosslinking. This technology has potential applicability for identifying appropriate lectins for binding to glycoprotein-variant disease biomarkers, which can be useful in developing diagnostic lectin-based assays, such as lectin blotting, lectin array, and lectin histochemistry.

A-082

Comparison of Suitable Additives for the Reliable Diagnosis of Gestational Diabetes

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Background: The number of subjects with diabetes has risen all over the world during the last years. Women with gestational diabetes (GD) are at an increased risk of complications during pregnancy and at delivery. The stability of glucose is important to reliably detect GD and prevent maternal and fetal complications. The routine process from blood collection until analysis requires transport times to a lab of up to 48h. The aim of the study was to investigate two suitable additives used in commercially available tubes for glucose measurements in view of inhibition of glycolysis.

Methods: The study was done at ISALA Hospital (Netherlands) using VACUETTE NaF/K_3EDTA and VACUETTE FC Mix (citrate, EDTA and NaF) blood collection tubes. Pregnant donors who were healthy (n=19) or diagnosed with GD by 75g-Oral Glucose Tolerance Test (n=24) were recruited. Informed consent was given and the study was approved by EC Netherlands. Venous blood was drawn from each donor into eight tubes (four tubes each tube type). One tube of each type was spun directly after blood collection. Plasma was measured immediately after centrifugation to obtain initial values (fasting) using the Hexokinase method on a COBAS 8000 (Roche, repeatability VC 1%, total precision VC 1.7%). All other whole blood specimens were kept at room temperature (RT). For evaluation of glucose stability, the second tube of each type was spun and measured at 2h, the third at 24h and the last at 48h. Statistical evaluation was done by STATISTICA 12.

Results: Evaluation of all clinical results for glucose concentration and any deviations was based on the Allowable Total Error Table (for glucose 10%) by Data Innovations. Performance testing revealed a clinically significant difference between stored whole blood specimens spun and measured after 2h, 24h and 48h to initially spun tubes repeatedly measured at the corresponding time point. The highest deviations were -11.5%; -13.4% and -11.1%, respectively. That deviation is due to the incomplete inhibition of glycolysis by the enolase inhibitor NaF alone. Using the FC Mix tube containing citrate, EDTA and NaF leads to more accurate glucose measurements by preventing the initial significant drop of glucose up to 4-6h.

Conclusion: The FC Mix tube is more suitable for reliable determination of blood glucose, one of the most frequently measured analytes and of primary importance

in diagnosis, monitoring and therapy of GD. The stability of glucose in whole blood specimens drawn in FC Mix tube and stored up to 48h at RT was demonstrated to be superior to the tube containing NaF alone.

A-085

LC-MS/MS Analysis of Methylmalonic Acid in Plasma for Clinical Research

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Background: Vitamin B12 plays an integral role in critical biological functions such as DNA synthesis, formation of erythrocytes, and maintenance of the myelin sheath in the nervous system. In its role as a co-factor, Vitamin B12 aids the methyl-malonyl-CoA mutase catalysed conversion of methylmalonyl CoA to succinyl-CoA, with Methylmalonic Acid (MMA) as an intermediate in this pathway. MMA concentrations are elevated when there is insufficient Vitamin B12 to help catalyse this reaction, meaning that MMA is a candidate biomarker for clinical research investigations into the function of Vitamin B12. LC-MS/MS is an ideal platform to perform this analysis. Chromatographic separation is key in separating MMA from isobaric species, such as succinic acid, that can cause a significant concentration bias. To help facilitate adoption of this methodology, a simplified workflow is necessary to enable a high throughput of samples.

Methods: MMA purchased from Sigma Aldrich (Poole, UK) was used to create calibrators in 1% (w/v) Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS), and quality controls (QCs) in pooled plasma (BioIVT, UK). Additional controls at two MMA concentrations were purchased from Recipe (Munich, Germany). Plasma samples previously analyzed using an independent LC-MS/MS were obtained for method comparison. 100µL plasma samples were added directly to a Waters Ostro™ phospholipid removal 96-well plates. 25µL internal standard was added, mixed and then samples precipitated with 400µL 1% (v/v) formic acid in acetonitrile. Samples were mixed using a plate shaker for 5 minutes prior to elution under vacuum into a 2mL 96-well collection plate. Samples were evaporated to dryness and reconstituted in 60µL 1% (v/v) formic acid in water. Using an ACQUITY UPLC™ I-Class system, samples were injected onto a 2.1mm x 100mm ACQUITY UPLC CSH™ C18 1.7µm column with an in-line filter using a water/acetonitrile/formic acid gradient and quantified with a Xevo™ TQ-S micro mass spectrometer.

Results: The method demonstrated no significant carryover or matrix effects and was shown to be linear from 21 - 1270 nmol/L. MMA was chromatographically separated from succinic acid, increasing the selectivity and accuracy of the method. Extraction efficiency of MMA ranged from 87-95% at three concentrations across the calibration range. Coefficients of variation (CV) for total precision and repeatability on 5 occasions for low (152 nmol/L), mid (364 nmol/L) and high (982 nmol/L) concentrations of the pooled plasma QC material, and for level 1 (264 nmol/L) and level 2 (598 nmol/L) Recipe controls were all ≤8% (n = 25) for MMA. Investigations indicate the analytical sensitivity of this method would allow precise quantification (<20% CV) at 22 nmol/L with a Signal/Noise (S/N) > 10. Analysis of samples previously analyzed by an independent LC-MS/MS method demonstrated MMA concentrations were within 10% mean bias for the samples.

Conclusions: Successfully quantified plasma MMA using a simple protocol on the Ostro phospholipid removal plate followed by LC-MS/MS analysis, for clinical research purposes. The method demonstrates excellent sensitivity, linearity, precision and bias with minimal matrix effects.

For Research Use Only, Not for use in diagnostic procedures.

A-087

Serum Trimethylamine N-oxide Stability and Its Correlation with the Severity of Coronary Atherosclerosis

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Background: Cardiovascular disease (CVD) has become a global threat to human health, Beyond traditional residual risk indicators such as blood glucose, lipid profile and so on, an increasing number of studies have been focused on the discovery of new risk predictors. Trimethylamine N-oxide (TMAO), generated from dietary choline, betaine and L-carnitine, is one of the most discussed gut metabolites since it plays an essential role in atherosclerosis (AS) which is a common pathological basis for many CVDs. Some studies have described the relationship between TMAO and AS from different perspectives, but in clinical application, the stability of serum TMAO, the distribution characteristics of Chinese apparent healthy population, the relationship between TMAO and the severity of coronary heart disease(CHD)shown by coronary angiography are all need to be further studied and elaborated. These problems will be solved in this study. **Methods:** TMAO was detected by liquid chromatography tandem mass spectrometry (LC-MS/MS), which has been evaluated strictly and can meet the clinical application standard. ① Stability evaluation: six samples with different concentration covering linear range were stored under different conditions for different periods: room temperature for 2hours, 4hours and 8hours ; 4℃ for 1day, 2days and 3days; - 20 ℃ for 1day, 2days, 3days,15days and 30days. To evaluate the stability of samples after treatment, pretreated samples were stored at 10℃ and room temperature for 24 hours and 48 hours respectively, variation less than 15% was considered acceptable . ② Distribution of TMAO in healthy population and its relationship with the severity of CHD: 236 cases of apparent healthy population and 266 patients with CHD were enrolled in our study. Correlation between TMAO and Gensini score was analyzed based on coronary angiography. **Results:** ① Stability: Serum TMAO can be stable for at least 8 hours at room temperature(2.9%), 3 days at 4℃(-3.43%) and 30 days at -20℃(-10.67%) respectively,TMAO eluent which was pretreated in advance can be stable for at least 48 hours (less than -1.05%)at room temperature or 10 ℃. The average variation of serum TMAO after freezing and thawing for three times is not higher than 5.4%. ② In apparent healthy population, the reference interval of serum TMAO was 0.038-0.378ug/ml, and serum TMAO of healthy people [0.130(0.09,0.182)µg /ml] was lower than that of the patients with CHD [0.180(0.126,0.278)µg/ml],(P<0.001).Weak correlations between TMAO and age(r=0.154,P<0.01),urea nitrogen(r=0.186,P<0.01),glucose(r=0.163,P<0.01),uric acid(r=0.115,P<0.01)were found,while there was no correlation with lipid profile. Serum TMAO gradually increased from the second quartile to the fourth quartile of Gensini score,however,It was found out that serum TMAO exhibited no independent predictive value for the occurrence of CHD after adjusting for traditional risk factors(gender,age,Glu,LDL-C). **Conclusions:** As one of the important members of intestinal metabolites,serum TMAO has a certain correlation with the interquartile of Gensini score, but whether it can be used as a CHD prediction index independent of traditional risk factors is questionable.**Key words:** Trimethylamine N-oxide, Liquid chromatography tandem mass spectrometry (LC-MS/MS), Stability, Coronary atherosclerosis

A-088

Saving Lives through Laboratory-Clinical Interface: The Case of Pseudohyperkalemia in a Patient with CLL

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Background: Pseudohyperkalemia defines a clinical situation in which high serum or plasma potassium level does not reflect the true in vivo level. Pseudohyperkalemia can result from pre-analytical procedures like: difficult venipuncture, transport, storage of specimens, and pathophysiological reasons like: leukocytosis and thrombocytosis among many other. Due to a low incidence, pseudohyperkalemia is difficult to confirm in patients with chronic lymphocytic leukemia (CLL) in whom leukemic blast cells are fragile and prone to lysis by process like venipuncture or centrifugation. Pseudohyperkalemia should always be excluded before implementing treatment that could derivate in hypokalemia or potentially fatal outcomes. Our report describes a case showing the importance of confirming Pseudohyperkalemia in time.

Methods: A 78-year male with CLL was admitted to the emergency room with deteriorating state of fatigue, dyspnea, hepatosplenomegaly and severe anemia with leukocytosis. He was referred to internal medicine department.

Results: The laboratory test result for plasma potassium concentration (K-B) was 7.2 mmol/L (lithium heparin with gel separator) (NV: 3.5-5.0) no hemolysis was detected in the sample, urea and creatinine were within the normal range (NV), white blood cell (WBC) count was159.2 x109/L (NV: 4.0-11.0), and platelet count was (PLT) 170 x109/L (NV: 140-400). The patient underwent initial therapy for hyperkalemia (for 3 days). During these three days, the laboratory tests results were as followed: K-B (mmol/L): 6.4, 9.2, 5.0, 5.2, 11.3, 5.8, 5.8, 5.3, (no hemolysis, urea and creatinine within normal range); WBC (x109/L): 159.2, 141.4, 195.2, 142.8, 182.3, 205.3, 184.5, 189.2, 166.5, and 157.8; PLT (x109/L): 170, 180, 184, 173, 163, 168, 151, 139, 143. Since plasma potassium level did not reach normal values, the medical resident contacted the laboratory to enquire whether the laboratory equipment was functioning adequately. The laboratory recommended drawing a whole blood sample in heparinized syringe to measure potassium concentration (K-WB) in order to rule out possible pseudohyperkalemia. Venous blood gas collected (by the same resident), was sent to the laboratory (not by the pneumatic system) and K-WB level was 2.6 mmol/L demonstrating current state of hypokalemia and confirming pseudohyperkalemia The

initial treatment aiming at lowering potassium level was immediately discontinued. A day after, the patient returned to Normokalemia with K-WB 3.6 and K-B 9.4 (pseudohyperkalemia) ratifying an absence of electrolyte imbalance.

Conclusion: It is crucial for the treating physician to be aware of the pseudohyperkalemia phenomenon, especially when leukocytosis is present. Furthermore, the laboratory professional who reports leukocytosis should always consider a false high potassium concentration result, adding a comment to the result of potassium concentration that indicates the possibility of pseudohyperkalemia. It is also very important to alert the physician in case of Pseudohyperkalemia, about the necessity to measure potassium level in heparinized blood gas syringe sample, avoiding the use of the pneumatic system for delivering the sample, in order to eliminate extensive blasts destruction.

A-089

Evaluation of Clinical Chemistry Assays of Deficiency Anemias Basic Profile using Sigma Metrics in a Clinical Laboratory in Brazil

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Introduction: Anemia affects about 2 billion people globally, being one of the most relevant problems in developing countries, such as Brazil. Anemia is characterized by a low concentration of hemoglobin in the blood and a drop in red blood cells and hematocrit counts, however, these parameters occasionally show normal levels in patients with hemoglobin depletion. According to the 2006 National Demography and Health Survey (NDHS), iron-deficiency anemia reaches 20.9% among children under 5 years old, 24.1% in children under 2 years old, and 29.4% of fertile women. The authors of this study aimed to analyze Iron (Iron), Transferrin (Trf), Total Iron Binding Capacity (TIBIC), Ferritin (Fer), Folate (FO) and Vitamin B12 (VB12) assays using Sigma Metrics on Atellica CH and Atellica IM analyzer, to evaluate the performance of these parameters in a large laboratory in São Paulo, Brazil. **Methods:** The precision evaluation and bias estimation by peer group was performed through the repeatability study (%CV_R) and within-laboratory precision (%CV_{WL}), according to EP15-A3, with a total of 25 samples per QC. The evaluation criteria was based on the comparison of the coefficient of variation obtained with the manufacturer and Total Allowable Error (TEa) specifications. Comparison studies were performed for TIBIC on the Atellica CH and AU 5800 Beckman Coulter Analyzers and for Ferritin, Folate and VB12 on the Atellica IM and Abbott Architect Analyzers according to EP09 using, at least, 50 serum samples. In the Sigma metrics evaluation, precision and bias components were used for each level of QC. **Results**: The precision results agree with the analytical quality specifications. The CV_R obtained was 0.6% to 2.7% and CV_{WL} was 0.6% to 3.6%. Using the TEa based on the Biological Variation table, 8 of 10 results showed 6 sigma performance (world class) and 2 of 10 showed sigma between 3 and 5.9 (good). Using RCPA as reference, 4 of 10 results showed 6 sigma and 6 of 10 with sigma performance between 3 and 5.9 (good). **Conclusion**: All assays tested on the Atellica CH Analyzer have demonstrated acceptable results of precision study (consistent with analytical quality specifications) and sigma metrics, which can provide a basis for understanding the performance of the Atellica Solution Assays. Despite conducting the study of sigma metrics in this work, it is worth mentioning that there is still no consensus on the appropriate source of TEa and how the bias should be calculated or what is the relevant analyte concentration for a given assay, which makes sigma metrics results of complex interpretation. However, these results generate confidence to use Atellica Solution Assays after implementation. **Siemens Healthineers supported the studies by providing systems, and reagents.*

A-091

Performance Evaluation of Liver Profile and Lipid Profile Assays on Two Platforms of Atellica CH 930

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Introduction: There is an increasing recognition of the need to evidence the quality of the analytical method and the equivalence between two or more platforms in the same segment, whether in the implementation of new assay or in exchange for analytical platforms. **Objectives**: Evaluate the analytical performance of the Albumin, Total Protein, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma Glutamiltransferase (GGT), Cholesterol, Triglycerides, HDL Cholesterol, LDL Cholesterol assays by verification of the imprecision study, method comparison and the evaluation of equivalence between two Atellica CH 930 Biochemistry Analyzers. **Methods:** Verification of Imprecision was trough repeatability (%CV_R) and within-laboratory imprecision (%CV_{WL}) according to EP15-A3, comparison of methods according to EP09-A3. For imprecision study, two concentrations were used; each level of QC materials was tested in one run per day for five days, resulting in a total of 25 replicates per sample for each assay. Method comparison studies were performed using at least 20 serum samples that covered the assay range. The equivalence evaluation was performed by evaluating the precision of each assay in both Atellica CH analytical systems and to determine the inter equipment performance was adopted the target value deviation Index (Z), against of the TEa metrics. **Results**: Precision results are in accordance with analytical quality specifications (AQS) ranging from 2.4% to 9.7%. The %CVR was from 0,31% to 3,48% and %CV_{WL} was from 0.39% to 3.73% for all Atellica CH assays. Method comparison results R2 0,986 to 0,999. We observed that 100 % of the differences are within the calculated ETa for all trials. Assays tested on the Atellica CH Analyzer and ADVIA Chemistry demonstrated optimal agreement. The two analytical systems are equivalent because the highest CV observed was less than twice the lowest CV observed for each equipment and the difference between the observed averages was less than the BIAS specification. **Conclusion**: All assays tested on the Atellica CH 930 Analyzer have shown acceptable precision results, comparison and equivalence. The study demonstrated that there is no clinical impact n the trials tested on a possible change to Atellica CH 930.**Siemens Healthineers supported the studies by providing systems, and reagents.*

A-092

Biomarkers of Mild Traumatic Brain Injury (UCH-L1, GFAP) and Age: An Analysis Using Regression Modeling

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Background: Serum glial fibrillary acidic protein (GFAP) and ubiquitin carboxyl-terminal esterase L1 (UCH-L1) have recently received US Food and Drug Administration approval for prediction of abnormal computed tomography (CT) in mild traumatic brain injury patients (mTBI). However, the factors that influence their performance have not been fully characterized. Previous studies by this group have shown elderly aged patients exhibit higher biomarker values in conjunction with negative CT findings Accordingly, the assay specificity was lower in elderly patients compared to younger patients. Our objective was to model the influence of co-variates including age, UCH-L1/GFAP on its companion biomarker, using either UCH-L1 or GFAP as the dependent variable in a multiple linear regression model. **Methods:** We performed a post-hoc analysis using study data from ALERT-TBI: A Prospective Clinical Evaluation of Biomarkers of Traumatic Brain Injury, a multi-center study designed to validate the Banyan Brain Trauma Indicator (BTI™) strategy to detect intracranial injuries within 12 hours of trauma. Previously recorded mTBI patient variables including age and serum values of GFAP and UCH-L1 from mTBI patients were modeled using multiple regression analysis. **Results:** By using UCH-L1 as a dependent variable, we created a multiple regression model showing a positive correlation between UCH-L1 vs age (β=2.012, $p<0.0001$), and UCH-L1 vs GFAP (β=2.685, $p<0.0001$). A similar model using GFAP as the dependent variable, was not well described by both age (β=0.138, p=0.0566, ns) and UCH-L1 (β=0.0567, $p<0.0001$). When we re-analyzed these data by partitioning the patient population into sub-groups based on positive or negative CT findings, the influence of the biomarkers and age followed a similar outcome. **Conclusions:** Regression models may be effective for describing UCH-L1 (dependent variable) in relationship to age and GFAP. However, similar modeling with GFAP are not effectively predicted by age and UCH-L1. For GFAP, this is likely due to the insignificant contribution of age as an independent variable in these models. Other factors, including episodic and clinical variables, as well as practical considerations may better predict the biomarker responses recorded in the Banyan biomarkers, especially with regards to elderly patients.

A-093

Comparing Turbidimetry and Nephelometry Precision in Automated Analysis of Urinary NGAL

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Background: Urinary neutrophil gelatinase-associated lipocalin, The NGAL Test™ (BioPorto Diagnostics A/S), is assayed in our hospital lab, to evaluate risk of acute kidney injury (AKI) in children. We evaluated precision of the Siemens Healthineers Dimension Vista® (nephelometry) and Siemens Healthineers Atellica® CH Analyzer

(turbidimetry) as we were transitioning from Vista to Atellica in our main lab. **Methods:** The mean and percent coefficient of variation were calculated from 10 within-run assays of 14 pediatric urine samples that spanned the analytical measurement range (AMR). **Results**: The value and % cv are shown in the table below. We adjusted our lower analytical limit from 25 ng/mL that we had formerly determined for the Vista, to 50 ng/mL for the Atellica, based upon this analysis. Monthly precision, based upon quality control samples, was similar between Vista (3.64% cv for 196.6 ng/mL and 3.04% cv for 503.3 ng/mL) and Atellica (2.1% cv for 203.3 ng/mL and 1.6% cv for 511.1 ng/ml). **Conclusion:** The turbidimetry method of the Atellica, while less precise than the nephelometry method in the Vista, is acceptable for clinical use.

Urinary NGAL mean (ng/mL) and % cv			
Vista value	Vista % cv	Atellica value	Atellica % cv
41	4.7	49	20
85	12.1	95	14.4
125	6.0	132	7.5
254	1.6	262	6.2
305	2.4	313	4.8
418	1.4	430	4.6
740	1.7	759	1.9
874	0.8	879	3.7
1189	0.9	1201	1.7
1260	1.0	1283	1.9
1570	1.1	1432	1.6
2239	1.3	2145	2.1
2384	1.5	2386	2.4
2560	2.0	2586	3.5

A-094

Evaluation of Lipemia Interference Cutoffs for Sodium by Indirect Ion-Selective Electrode

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Background: Lipemia is a common pre-analytic interference that can cause erroneous results usually due to altered light scattering of a sample. In the case of pseudohyponatremia, however, extreme hyperlipemia is understood to cause spuriously low concentrations of sodium due to an altered ratio of water to dissolved solids in serum and plasma, commonly known as the electrolyte exclusion effect. Despite using the manufacturer provided lipemic cutoff (L-index=2000), cases of lipid-induced pseudohyponatremia were still identified at much lower lipemia values. As such, we evaluated the ability of Intralipid to induce pseudohyponatremia in vitro and sought to establish a new lipemia cutoff interval for indirect ion-selective electrode (ISE) sodium using actual patient specimens. **Methods:** Three plasma pools, severely hypo-, mildly hypo- and normonatremic, each with L-indices <10 were separated into 275uL aliquots to which 25uL serially diluted Intralipid (with water) was spiked to achieve L-indices ranging from approximately 2000 to <10. The sodium concentration by indirect ISE (Cobas c702; Roche Diagnostics) and L-index of each aliquot was determined. Pseudohyponatremia was evaluated in naturally hypertriglyceridemic plasma samples by comparing pre- to post-ultracentrifugation sodium measured by indirect ISE (Cobas c702). By using ultracentrifugation, rather than a direct ISE method, we were able to interrogate only those differences in sodium caused by lipids, excluding other common factors such as total protein. A sodium change ≥4 mmol/L was considered significant interference. **Results:** Plasma pools with sodium concentrations at 114, 125, and 140 mmol/L were used for the Intralipid spiking evaluation. Intralipid did not induce a significant change at any concentration tested, with mean sodium differences from baseline (mmol/L (range)) of -1 (-2-0), 2 (1-3), and 0 (-1-2) for the severely hypo-, mildly hypo- and normonatremic pools, respectively. Pseudohyponatremia was evaluated in plasma samples (n=16) with hypertriglyceridemia (413->4425 mg/dL, L-index 18-1288). Pseudohyponatremia was identified in 2 samples. The first sample had an L-index = 1288, triglycerides >4425 mg/dL, and a sodium change (pre- to post-ultracentrifuged) of 120 to 131 mmol/L. The second sample had an L-index = 481, triglycerides = 835 mg/dL, and a sodium change (pre- to post-ultracentrifuged) of 126 to 137 mmol/L. **Conclusion:** The naturally hypertriglyceridemic plasma samples demonstrate that pseudohyponatremia can occur at lipemia levels well below the manufacturer cut-off of 2000. Using Intralipid to evaluate lipemia interference is a common practice, however, Intralipid may not be suitable for use in the determination of lipemia cut-offs for sodium because it does not exhibit the same electrolyte exclusion effects seen in some lipemic patient samples. Most hyperlipemic/hypertriglyceridemic patients in this study did not demonstrate pseudohyponatremia, which indicates the electrolyte exclusion effect is variable and likely dependent on the individual patient. While a lipemia cut-off of <481 would identify both pseudohyponatremic patients in this study, the specificity for pseudohyponatremia would be low and the sample number used to derive that cutoff is small. Laboratories should be aware of the potential for pseudohyponatremia at L-indices lower than described by studies using Intralipid as a surrogate for human-derived lipids.

A-095

Development of Liquid-Stable Calibration Verification Sets for Fertility Markers to Characterize Method Linearity and to Verify the Reportable Ranges.

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Introduction**:** There are a number of assays available for testing on automated chemistry analyzers to, monitor and evaluate patient growth, sexual development, fertility status and disease states across all ages and sexes. Clinical laboratory tests commonly used include; alpha-fetoprotein (AFP), dehydroepiandrosterone sulfate (DHEAS), estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), prolactin (PRL), progesterone (PROG), testosterone (TST), Anti-Müllerian hormone (AMH), and sex hormone binding globulin (SHBG).

Each of these analytes may be tested for different reasons depending on the sex of the patient. For example, in males, TST is responsible for development of masculine sexual characteristics, regulation of sex drive, and muscle mass. In males low amounts of circulating TST are converted to E2. In females, TST is produced by the ovaries and is converted to E2. Imbalances in TST can affect fertility, with low levels in males and high levels in females linked to infertility. SHBG binds to TST carrying the hormones throughout the body in our blood. E2 is a steroid responsible for the development of female sexual characteristics and although present at low levels in males, its role in male fertility has not been fully characterized. In females, E2 works with PROG regarding development of the uterus and breasts, regulation of the menstrual cycle, and is important for maintaining a healthy pregnancy.

The most common fertility testing done in males is a sperm count and motility analysis. If counts are low, blood tests for analytes such as TST, PRL, LH, SHBG and FSH are ordered. Fertility testing in females commonly includes blood tests for LH, FSH, PRL, E2, PROG, and AMH. For this reason, LGC determined that it was necessary to offer an expansive product line containing these fertility analytes in Fertility 1 (502RE), Fertility 2 (504RE), and Fertility 3 (currently in development).

Methods: Each product in the VALIDATE® Fertility line is formulated as a multi-constituent kit, in a human serum matrix, according to CLSI EP06-A into five equal-delta concentrations to cover the reportable ranges of each analyte on the Roche cobas®. For each level, samples were tested in triplicate for each of the analytes. Reported recoveries were evaluated for mean, SD and linearity using MSDRx®, LGC's proprietary linearity software. Limits were applied as a percentage of the total allowable error (TE_a), specific for each analyte. Product stability was determined by a combination of stress and real-time stabilities.

Results: For the VALIDATE® Fertility line, 502RE and 504RE, all levels are stable for their claimed expiration and evaluate the assay's linearity through the manufacturers' reportable range. The VALIDATE® Fertility 3 formulation is in development and studies are on-going. Conclusion: The currently available Calibration Verification test kits are listed with the FDA, CE marked and aid in testing and documenting of calibration verification, analytical measurement range verification and linearity which are required by many inspection agencies. The products also support the user when troubleshooting instrument systems, reagent problems and calibration anomalies.

A-097

The Clinical Importance of Including P-5'-P In AST and ALT Methods

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Case Report: A 62-yr-old man arrived at our ER by ambulance at noon after a fall from a 9-ft ladder. He is an air force veteran with a history of PTSD and polysubstance abuse (alcohol, cocaine, and marijuana). Vital signs showed a normal temp, respiration rate, O_2 sat, blood pressure, and pulse. His height was 5'11"; weight was 68 kg

(150 lb). The patient reported alcohol use prior to the fall, including a fifth of vodka and a 12-pack of beer the night before, and another beer at 8 am. Pertinent lab results Pertinent lab results include glucose 65 mg/dL, protein 4.6 g/dL, ethanol 144 mg/dL, AST < 3 U/L, and ALT < 3 U/L. The patient was alert, oriented, and regarded as clinically stable. The clinicians noticed the AST and ALT results of < 3 U/L and initially concluded that the patient had burned out his liver, but then realized that this option did not make sense in view of other lab results and clinical signs. They called us to convey their confusion. We investigated.**Background**: AST and ALT are determined on Beckman Coulter AU platforms (5800 & 680) in our laboratory. Pyridoxal-5'-phosphate (P-5'-P, vitamin B_6) is a necessary coenzyme for amino-transferase reactions. Patients with nutritional disorders or similar health issues will be deficient in P-5'-P. For clinical enzyme assays, all factors affecting the rate of reaction should be optimized. The addition of P-5'-P in aminotransferase methods is therefore recommended to ensure that all enzyme present in the sample is measured. We logically assumed the methods we used had been optimized accordingly. But we could not confirm that fact by reviewing the procedures. Contacting colleagues at Beckman produced the following reply: "P-5-P (pyridoxal phosphate) is not available for ALT and AST in the US."**Response**: 1.The IFCC recommended that AST and ALT methods include P-5'-P in the reagents over 40 years ago.2.P-5'-P is, in fact, available for these reagents in the US, and is an option for customers of Siemens and Roche.3.We found out by contacting laboratory professionals through social media that the US market favors these reagents without P-5'-P supplementation due to convenience, reagent stability, and assay linearity. Many users, however, may have no idea that their AST and ALT methods lack P-5'-P supplementation and thus potentially produce falsely low values.4.Our patient's low AST and ALT results were probably due to poor nutrition. The clinicians taking care of this patient proceeded accordingly. **Conclusion**s: Incorrect patient results for AST and ALT are being reported from laboratories using inferior, unsupplemented methods. In our laboratory, anywhere from 6-10 samples with AST and/or ALT values < 3 U/L are seen each month. Doctors are making incorrect conclusions on an unknown number of their patients. These doctors may not contact their clinical laboratory colleagues but are puzzled by such results that don't match the clinical picture. Care providers should be encouraged to interact with laboratory professionals to avoid frustration and confusion.

A-099

Establishment of a Quick Method to Quantify Urinary Porphobilinogen and Reference Intervals Using Retrospective Laboratory Data

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Background: The porphyrias are a class of heterogeneous diseases of heme metabolism characterized by neurovisceral symptoms in acute porphyria and cutaneous photosensitivity in cutaneous porphyria. Elevation of urinary porphobilinogen (PBG) is characteristic of acute porphyrias, which include acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria, and are distinguished by acute attacks that can produce neurologic damage and possible death without timely intervention. Current methods for measuring PBG include ion-exchange followed by reaction with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) and spectrophotometric detection, and tandem mass spectrometry. While these methods are clinically valid, manual methods are laborious, time-consuming, and prone to interferences, and mass spectrometry is more costly. Furthermore, the reference intervals for PBG are historical and are not normalized to creatinine for a robust interpretation of random urine collection. Our objectives were to (a) develop a fast and easy-to-perform method to quantify urinary porphobilinogen, and (b) establish random creatinine-normalized and 24-hour excretion reference intervals for urinary porphobilinogen using retrospective laboratory data. ***Method:*** We adapted the method of Roshal *et al.* (*Clin Chem* 54:429) using sodium acetate-conditioned anion-exchange resin packed in a spin column to separate PBG from interfering substances. Samples were applied to the column and washed twice following centrifugation. PBG was eluted with acetic acid. The eluate was mixed with Ehrlich's reagent, and the concentration of PBG was measured spectrophotometrically at 555 nm after 10 minutes incubation. The linearity of the method was assessed by testing six concentrations of PBG, selected to span the analytical measurement range of the assay, in triplicate for 4 days. An imprecision study was performed by analyzing duplicates of level I and level II controls in 5 runs completed on 5 different days. We compared our new method to the method in-use by testing previously analyzed urine samples. To establish reference intervals, 10 years of de-identified retrospective PBG results, measured by the anion-exchange column method, were obtained from the laboratory information system. The Hoffmann approach was used to calculate reference intervals for PBG normalized to creatinine and as daily excretion. ***Results:*** Accuracy and linearity investigation produced a reportable range of 0.5 to 50 mg/L, slope of 0.99, intercept of -0.01, and observed error of 4.6%. Within-run imprecision (CV) for the low and high controls at mean concentrations of 2.0 mg/L and 20.0 mg/L was 5.4% and 4.4%, respectively, and between-run imprecision was 5.5% and 4.3%, respectively. Comparison with our current method produced a Deming regression slope of 1.2, a correlation coefficient of 0.99, and a bias of -3.6%, with approximately 100% recovery. The reference interval for urinary PBG for random collection (n=5980) was 0-1.6 mg/g of creatinine, and for 24-hour excretion (n=3033), 0-2.4 mg/day. ***Conclusion:*** We developed an easy-to-perform and quick method to measure urinary PBG. The test compared well to current PBG testing methods. We established reference intervals for PBG normalized to creatinine in random specimens and for daily urinary PBG excretion. Our method and reference intervals will facilitate the rapid screening of acute porphyria to enable prompt intervention.

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Performance and Sigma Metrics Evaluation in Special Protein Assays on the Atellica CH 930 Analyzer

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Introduction: A laboratory method must fulfill some basic requirements that guarantee the reliability of the results obtained in patient samples. New methodologies, when implemented in the clinical laboratory, need to have the performance parameters of the method or equipment evaluated. That requires experimental studies to estimate whether the practical findings can support decision-making, as well as to verify if their performance meets the needs and expectations of the physician requesting the exam. Currently, one of the most used methodologies to evaluate the processes performance is the analysis with sigma metrics. The objective of this work was to evaluate the performance of 12 Special Proteins assays during a turbidimetry platform implementation in a clinical laboratory in São Paulo - Brazil, where nephelometry was the previously used methodology. **Methods**: Assays of Alpha Glycoprotein Acid (AGA), Apoliprotein-B (APO-B), Anti-streptolysin O (ASO), C3, C4, C-Reactive Protein (PCR), IgA, IgG, IgM, Lipoprotein (a) (Lp (a)), Rheumatoid Factor (RF) and Transferrin (Trf) were evaluated in the Chemistry Analyzer Atellica CH through the imprecision study, bias estimation and sigma metrics. Precision was assessed by the repeatability study ($\%CV_R$) and within-laboratory imprecision ($\%CV_{WL}$), according to EP15-A3, with a total of 25 replicates per sample for each assay. The bias estimation of the measurement procedure was compared to the peer groups, and the bias and total error was calculated for each quality control (QC) level. The evaluation criteria was based on the CV% comparison obtained with the manufacturer and Total Allowable Error (Tea) metrics. For the sigma metrics evaluation, the bias, imprecision study and the reference of Biological Variation 2014 for ETa were used. **Results:** Precision results agree with the analytical quality specifications. It was obtained a $\%CV_{WL}$ of 0.000% to 8.196% and $\%CV_R$ of 0.000% to 3.452%. When evaluating the performance of each level of QC by sigma metrics, approximately 91.7% of the levels presented satisfactory performance with sigma above 3, and AAG, C4, PCR, IgA and IgG assays obtained a Six Sigma level (world class) at all tested QC levels. The assays that obtained a sigma level below 3 were two levels, the IgM level 1, with a very close sigma of 2.9, and the Lp (a) level 1, in which the Peer Group data variations were observed depending on the reports versions, which may bring limitations in sigma interpretation. **Conclusion**: The results of sigma metrics were satisfactory in 91.7% of the QC levels and all tests showed acceptable results of imprecision and bias, consistent with the analytical specifications. Even conducting sigma metrics analyses in this study, there is still no consensus on the ETa proper source and it was possible to identify variations in the data found from the Peer Group report, making sigma metrics a tool which the interpretation must consider all these variables. This methodology, however, can be implemented in the laboratory for periodic evaluations, in order to know the assays performance in the new technology and to guarantee confidence in the results. **Siemens Healthineers supported the studies by providing systems, and reagents.*

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Performance Evaluation of Glycated Hemoglobin Assays on Different Analytical Platforms

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Background: Diabetes Mellitus affects more than 420 million adults worldwide, according to studies by the World Health Organization, and the costs associated exceed 727 billion dollars. The glycated hemoglobin assay (HbA1c) can be used in the diagnosis and monitoring of diabetic patients because it represents the patient's glycemic status between 2 to 3 months. After multicenter studies related small changes in the levels of HbA1c with the prognosis of patients and micro and macrovascular complications, it became necessary to standardize the different methodologies used for the quantification of HbA1c, ensuring that the variations in results reflect, in fact, clinical and not analytical variations. Therefore, this work aimed to evaluate the performance of three different methodologies for the quantification of glycated hemoglobin on four different platforms, comparing the turbidimetry and enzymatic methodology with the reference methodology (HPLC) and examine hemoglobin variant interference. **Methods**: The performance evaluation of the HbA1c assay included precision, correlation and variant hemoglobin interference assessment. Correlations were made with Atellica CH, Dimension ExL, Variant II and Premier Hb9210. The precision was verified through a repeatability study ($\%CV_R$) and within-laboratory precision ($\%CVW_L$) according to EP15-A3 with QC and four medical decision pools (MDP), and the method comparison according to CLSI Document EP09 using at least 100 whole blood samples that covered the entire linearity range of the assay. The interference study was performed with 10 samples from the four main types of variants (HbC, HbD, HbE and HbS). **Results:** The accuracy results are in accordance with the NGSP quality specifications (CV <3.5%). For the Atellica CH enzymatic assay, the $\%CV_R$ was 0.638% to 1.842%, and the $\%CV_{WL}$ was 0.631% to 2.331%. %. For the Dimension EXL Hemoglobin A1C assay (A1C), a turbidimetric assay, %CVR was 0.555–0.990% and %CVWL was 0.620–1.292%. The comparison study with HPLC resulted in a Pearson Coeficient of 0.9934 for Atellica CH A1c_E assay and 0.9909 for Dimension EXL A1C assay when compared to VARIANT II, and resulted in a Pearson Coeficient of 0.9901 for Atellica CH A1c_E assay and 0.9810 for Dimension EXL A1C assay when compared to Premier Hb9210. In the interference study, the

samples contained from 5.2% to 42.8% of variant hemoglobin and when compared to Premier Trinity, a bias of -1.64% to 4.43% for Atellica CH and -4.48 to 3.39 for Dimension ExL was obtained for HbS and HbC variants, the most common types in Brazil. **Conclusion:** Both the Atellica CH enzymatic and Dimension ExL turbidimetric assay presented acceptable results of imprecision and bias, being consistent with the analytical quality specification suggested by the NGSP, which is essential to ensure the safety in the use of these methodologies in the diagnosis and monitoring of diabetic patients beyond the methodology considered as reference (HPLC). **Siemens Healthineers supported the studies by providing systems, and reagents.*

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Demonstration of a Highly Purified Type-A Hydrolyzed Collagen Product as an Exceptional Protein Stabilization Matrix

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Background: The preservation of biological activity of protein preparations is critical for applications in the IVD industry. Protein standards are used by clinical laboratories to verify the performance of assay systems, ultimately ensuring accurate patient diagnoses.

Protein/peptide biomarkers are formulated to help maintain their native three-dimensional structure in-vitro to ensure accuracy and reproducibility on assay systems. Human and Bovine Serum Albumins (HSA/BSA) have both been used as protein stabilizers. The mechanisms of stabilization may be due to a combination non-covalent electrostatic interactions and competitive protection from adsorption loss and proteolytic degradation.

Gelatin, a byproduct of partially hydrolyzed collagen (HC), is also used as a protein stabilizer and excipient. Prionex® (DSM Pentapharm, Switzerland) is a unique preparation of porcine derived type-A HC that is marketed as an alternative to serum albumin for various applications, including in-vitro protein stabilization.

Objective: To demonstrate the mechanism of protein stabilization properties of Prionex®: a highly purified porcine derived type-A hydrolyzed collagen.

Methods: Prionex is produced according to a procedure wherein porcine skin is treated with pepsin, purified, and subjected to mild acidic hydrolysis at elevated temperature to yield a non-gelling HC product with an average MW of 20 kDa.

A proprietary control formulation adjusted to 10 ng/mL with Troponin I-C and 200 pg/mL BNP were prepared in HSA, Prionex, and an equal blend, each to a 5% total protein content. Samples were incubated for 6 days at 25°C and were assayed for Troponin recovery on the Siemens Dimension RXL and for BNP recovery on the Abbott Axsym.

Results: After 6 days at 25°C, Troponin and BNP recovered as follows: 80% and 55% in HSA; 93% and 100% in Prionex; 96% and 89% in the blend.

Conclusions: The results confirm findings published by Gaffney (1996) that demonstrate the effectiveness of HC to preserve the biological activity of two very different proteins: a large multimeric protein of 42.3 kDa (Troponin I-C) and a short 32-amino acid peptide hormone (BNP).

Prionex dramatically improved the recovery of BNP, which is very susceptible to proteases. The thermal processing of Prionex inactivates viruses, prions, and contaminant proteolytic enzymes. Albumin has several ligand-binding pockets which can reduce the bioavailability of peptides. HC has been shown to remain structurally inert, lacking major secondary or tertiary structures that fold into binding pockets. The highly charged nature of the amino acid content may explain stabilization from electrostatic interactions.

Prionex also improved the recovery of Troponin I-C, particularly when blended with HSA. One study showed that collagen peptides of 8.5 to 20 kDa promoted HSA stability by retarding the thermal unfolding of tertiary structure and delaying aggregation. The blended matrix may function synergistically to preserve the confirmations of both HSA and Troponin complex.

Prionex has been shown to be an effective stabilizer of proteins with vastly different properties. The lack of enzymatic activity, inert structure, and charged amino acid content are likely mechanisms of stabilization. More than a pathogen-free alternative to albumin, Prionex is attractive as an exceptional protein stabilizer for many potential applications.

A-103

New Sentinel CH Parameter on Siemens Atellica CH Analyzer Closed Channel: Angiotensin Converting Enzyme (ACE) Evaluation Performances

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Background: In this study we evaluated the performance of the ACE Sentinel CH reagents on Siemens Atellica CH analyzer. Angiotensin converting enzyme (ACE, dipeptidyl carboxypeptidase) is a glycoprotein peptidyldipeptide hydrolase that cleaves histidylleucine dipeptide from angiotensin I, a relatively inactive decapeptide. The latter is converted to the potent vasoconstrictor, angiotensin II. ACE also inactivates bradykinin. Elevated levels of ACE activity occur in serum of patients with active sarcoidosis, and occasionally in premature infants with respiratory distress syndrome, in adults with tuberculosis, Gaucher's disease, leprosy, and in many other pathologic conditions involving lung and liver diseases. Reference values: 13.3 – 63.9 U/L. ACE hydrolyzes furylacryloylphenylalanine-glycilglycine (FAPGG) into furylacryloylphenylalanine (FAP) and glycylglycine. FAPGG hydrolysis results in a decrease in absorbance at 340 nm. **Methods:** Performance evaluation included Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ), linearity, intra-assay imprecision, inter-assay imprecision, on board reagent stability, lot calibration interval, instrument correlation and sample carry over following the current CLSI guidelines protocols. Data were evaluated using Microsoft Excel statistical tool Analyse-it. **Results:**

TEST	RESULTS
LoB	1st lot 2.4 U/L - 2nd lot 0.9U/L
LoD	1st lot 4.5 U/L - 2nd lot 2.9 U/L
LoQ	1st lot 8.4 U/L - 2nd lot 7.4 U/L
Linearity	1st lot linear up to 121 U/L - 2nd lot linear up to 126 U/L
Intra-assay imprecision	CV % 1.3% - 8.2%
Inter-assay imprecision	CV % 4.4% - 8.6%
On board and Pack Calibration Reagent Stability (up to 30 days + 10%)	Bias % from -9.8% to 10.0%
Lot calibration interval (up to 26 days, in progress)	Bias % from -7.1% to 7.1%
Instrument comparison (vs Beckman Coulter AU680)	Passing-Bablok fit: y = 0.92x + 3.74 U/L r = 0.984
Sample carry over	Bias% -2.03%; Bias% -0.55% (considering the gap between the two levels chosen to perform the test)

Conclusions: All tests showed acceptable results.

A-104

Method Development of Cholinesterase using Rat Duodenum and Ileum Tissues

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Background: Due to the use of organophosphates in pesticides, cholinesterase enzymes are often analyzed to diagnose exposure or poisoning caused by agricultural contamination to the environment. For this reason, preclinical research facilities design studies to find toxicity and efficacy in rat species using cholinesterase (ChE) analysis of many peripheral tissues. The ChE tissue testing must be a validated method for these GLP studies.

Objective: The purpose of the study was to demonstrate the transfer of validated ChE methods for measuring ChE in Sprague-Dawley (SD) rat brain, red cell, heart and diaphragm using a Siemens Advia 1800 chemistry analyzer, to the following SD rat peripheral tissues: duodenum and ileum

Method and Materials: Stock colony rats were euthanized by carbon dioxide inhalation and the following tissues were excised: duodenum and ileum. The duodenum had 10 cm collected starting 2 cm posterior to the stomach. The ileum had 10 cm collected starting 2 cm anterior of the colon. The intestines were cleared of any contents. The tissues were rinsed in 1% triton- X, dried and weighed before being snap frozen in liquid nitrogen. The samples were stored frozen at -70°C. Tissues for the cholinesterase analyses were all processed according to the validated ChE procedure for brain. All tissues were processed and analyzed within an hour. During processing the tissues were kept in an ice bath. Each tissue was diluted by 10 using 1% triton-X, homogenized, centrifuged for 15 minutes and analyzed on the Advia 1800 using Roche ChE reagent. All tissues were measured for a blank and ChE activity. The start and stop time for each step in the processing was logged. Each tissue underwent testing for linearity, intra-assay precision and inter-assay precision.

Results: It was found that the validated method for ChE was successful for use in the intestinal tissues. The linearity and intra and inter-assay precisions were successful after minor troubleshooting during testing that required additional tissues to be collected. The linearity of dilution for rat tissue ChE was considered acceptable for all tissues. The correlation coefficient was >0.9, the slope was within 0.7500-1.2500 and the %Nominal was within 75%-125%. Cholinesterase tissue activity intra-assay precision was considered acceptable on all tissues with the %CV ≤20%. The inter-assay precision was considered acceptable for all tissues tested over a 6-day period. The %CV was ≤20%.

A-105

Impact of Co-Morbidities on Temporal Changes 2-4 Hours after Presentation in Subjects with cTnI Values above the 99th Percentile

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Background: US manufacturers of high-sensitivity troponin (hs-cTnI) assays and the Fourth Universal Definition of Myocardial Infraction recommend the use of two criteria for diagnosis of Acute Myocardial Infarction (AMI): 1) one value exceeding the 99th percentile and 2) a temporal change in cTnI. In Europe, the absolute change in hs-cTnI values, independent of the 99th percentile, are used for AMI diagnosis, and used in categorical rule-in/rule-out algorithms. As these algorithms gain widespread use, it is important understand the impact for subjects with cTnI values above the 99th percentile and co-morbidities.

Methods: Subjects with suspected AMI were enrolled in the HIGH-US study. Subjects had blood collected at baseline and then at 2-4 hours. hs-cTnI levels were measured using the Siemens Atellica IM TnIH assay. AMI was adjudicated by an expert panel of cardiologists and emergency medicine physicians.

Results: 1942 subjects were enrolled with suspected AMI. 225/1942 enrolled subjects were adjudicated as AMI. 360/1942 subjects had hs-cTnI values above the 99th percentile. 168 (47%) of these subjects were adjudicated as non-AMI. 67% of subjects with hs-cTnI values above the 99th percentile had identified co-morbidities. The far-right column of the table shows ideal cut-offs for the absolute changes of hs-cTnI values for the cohort. The overall cut-off was >6ng/L. In subjects with hs-cTnI values above the 99th percentile and heart failure (row a) the cut-off increases to >18ng/L. The cut-off further increases to >34ng/L for non-heart failure subjects (rows b-e).

Conclusion: Clinicians should be aware that cut-off for the absolute changes in hs-cTnI, for AMI diagnosis, are ~five-fold higher in patients above the 99th percentile. Also, the absolute change AMI cut-off for hs-cTnI in heart failure subjects above the 99th percentile are ~three-fold higher than the total cohort.

	N		Baseline (ng/L) Median (25th-7th)		Change (ng/L) Median (25th-7th)		Ideal cut-off* (ng/L)
	AMI	*Non-AMI*	*AMI*	*Non-AMI*	*AMI*	*Non-AMI*	
Total cohort	225	1717	201 (78-617)	5 (2-14)	81 (10.9-377.4)	0.7 (0.3-1.8)	>5
All subjects with baseline cTnI value above the 99th percentile (a-e)	192	168	250 (120-944)	90 (59-183)	92 (13-474)	7 (3-17)	>19
a. Heart failure hospitalization/reduced LVEF	57	82	177 (76-488)	88 (59-188)	29 (6-196)	7 (3-16)	>18
b. Renal insufficiency (eGFR <60 mL/min/1.73m²)	30	33	371 (149-1072)	107 (69-178)	54 (13-500)	8 (5-12)	>34
c. Atrial fibrillation	11	5	123 (97-426)	109 (98-253)	73 (14-596)	10 (5-14)	>34
d. Infection, inflammation, cancer	10	10	218 (93-758)	58 (48-206)	264 (91-455)	6 (4-16)	>34
e. None/Unknown co-morbidity	84	38	313 (138-1236)	88 (55-155)	143 (22-605)	6 (3-19)	>34

* Ideal cut off for the absolute change in the cTnI value that minimizes false positives and false negatives. All subjects with an absolute change below the cut off would be considered negative for AMI

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Harmonization Potential of Six High-Sensitivity Cardiac Troponin Assays

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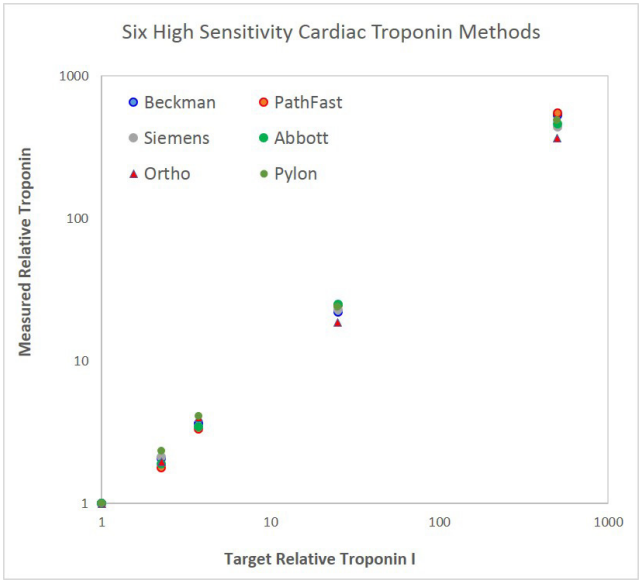
Background: Harmonizing cardiac troponin I (cTnI) measurements is complicated even in the era of high-sensitivity (hs)-cTnI assays. However, harmonization is critically needed because cTn is central to management of cardiac patients and guideline development. **Purpose:** Describe a study conducted by NIST and the IFCC workgroup for cTnI harmonization designed to assess the current state and potential for harmonization of hs-cTnI assays. Describe harmonization elements including method agreement, linearity and correlation of hs-cTnI methods for a set of five commutable samples spanning from slightly above the Limit of Quantitation (LoQ) to ~200-fold the male 99thpercentile Upper Reference Limit (URL). **Methods:** Pools of Li-heparin samples from acute myocardial infarction patients <72 hours after symptom onset were blended with normal hs-cTnI heparinized plasma to produce five commutable samples in the following hs-cTnI concentration ranges (Atellica IM TNIH values): S1. between LoQ and female 99thpercentile URL (~20ng/L); S2. male 99thpercentile URL (~54ng/L); S3. 30% greater than male 99thpercentile URL (~80ng/L); S4. 10-fold greater than male 99thpercentile URL (~500ng/L); and S5. 200-fold the male's

99thpercentile URL (~10,000ng/L). Sample sets were aliquoted into polypropylene screw-cap freezer vials and stored at -70^{0}C until measurement with six hs-cTnI assays; ARCHITECT STAT hsTnI (Abbott Diagnostics), ACCESS hsTnI (Beckman-Coulter), Atellica IM TNIH (Siemens Healthineers), hs Troponin I (Ortho), PATHFAST TnI-II (LCI Medisense), and Pylon cTnI (ET Healthcare). Each Method was normalized by dividing sequential (S2-S5) concentration by the lowest S1. hs-cTnI sample-value. Regression analysis of the hs-cTnI assays was performed. **Results:** Results of common commutable samples from each hs-cTnI method were plotted (Figure). Regression analysis yielded slope=0.942; y-intercept -0.1; correlation coefficient=0.990. **Conclusion:** Using a simple algorithm it was possible to harmonize six hs-cTnI methods. NIST and the IFCC cTnI Workgroup are developing Reference Material (RM8121), which is a concentration series of hs-cTnI samples for the purpose of harmonization of results.



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Evaluation of Harmonization across Siemens Healthineers Current Blood Gas and Clinical Chemistry Systems for All Common Analytes

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Objective: The objective of the study was to demonstrate harmonization for an end-to-end solution across the Siemens Healthineers current blood gas systems - the epoc® Blood Analysis System for patient-side testing, the new RAPIDPoint® 500e Blood Gas System* designed for the POC, the laboratory-based RAPIDLab® 1265 Blood Gas System, and the RAPIDLab 348EX Blood Gas System* designed for the lower volume testing sites - and the recently released central laboratory-based Atellica® CH 930 Analyzer. To prove harmonization and to minimize the misinterpretation of test results that may adversely impact patient outcomes, all common analytes were evaluated throughout the current point of care and laboratory diagnostics product portfolio.**Methodology:** A method comparison study was performed on the Siemens blood gas systems (epoc Blood Analysis System, RAPIDPoint 500e, RAPIDLab 1265, and RAPIDLab 348EX Blood Gas Systems) with whole blood versus the Atellica CH 930 Analyzer with plasma. The blood gas parameters (pH, pO_2, pCO_2) and ionized calcium (iCa^{++}) were evaluated versus the RAPIDLab 1265 system. The study design and analysis followed CLSI EP09c for the measurement of pH, pO_2, pCO_2, sodium, potassium, chloride, ionized calcium, glucose, lactate, creatinine, and BUN. Correlation statistics including regression type, slope, intercept, and coefficient of determination (r^2) were generated.**Validation/Results:** Correlation statistics for potassium are represented in Table 1. (Example of data presentation for the final poster)

Table 1. Method Comparison Statistics for potassium on the Siemens Blood Gas Systems vs Atellica CH 930 Analyzer

Comparison	n	Slope	Intercept (units)	r^2	Bias at MDL 1 (units)	Bias at MDL 2 (units)	Interval of x (units)
epoc system vs. Atellica CH 930 Analyzer	120	1.02	-0.08	0.997	-0.02	0.05	1.71 to 9.35
RAPIDPoint 500e system vs. Atellica CH 930 Analyzer	120	0.95	0.29	0.996	0.14	-0.01	1.90 to 9.35
RAPIDLab 1265 system vs. Atellica CH 930 Analyzer	112	1.01	0.03	0.993	0.06	0.09	1.54 to 9.35
RAPIDLab 348EX system vs. Atellica CH 930 Analyzer	104	0.99	0.21	0.994	0.19	0.17	1.54 to 9.35

Conclusion: Harmonization at the clinically relevant medical decision levels was demonstrated for a true end-to-end solution across all of the Siemens Healthineers blood gas and the recently released Atellica CH 930 Analyzer for all common analytes.

Note: Data for all analytes is presented in the poster as depicted in Table 1.

The products/features (mentioned herein) may not be commercially available in all countries. Not available for sale in the US.

A-109

Development of an Automated Enzymatic Assay to Measure Serum Aldolase Activity and Method Comparison using Two Calibration Approaches

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Background: Aldolase measurement have been of greatest clinical interest in primary diseases of skeletal muscle. Aldolase activity testing has been largely replaced with creatinine kinase (CK) although measurement of both aldolase and CK is considered suitable for diagnosing muscular dystrophies, neuromuscular disorders and skeletal muscle wasting. This test is offered at medical centers and reference laboratories. The College of American Pathologists summary from a recent ADL survey reports data from 75 participant laboratories, most of which utilize Roche reagents (Roche Diagnostics, Indianapolis, IN), followed by Randox (Randox Laboratories Ltd, Kernersville, WV) and CaldonBioTech (Carlsbad, CA), none of which is FDA approved in automated platforms. The Roche test utilizes a k-factor derived from molar extinction coefficients for quantitation in lieu of a calibration curve. The alternative use of a calibrator has the potential to improve the assay performance, particularly at low concentrations, which was not accomplished in a previous attempt in our laboratory to use a k-factor (data not shown). The use of a k-factor allows accurate analysis if all variables of a test are constant, whereas the use of a calibrator provides a known value based on the current variables of the assay. Our objective was to evaluate Roche's aldolase reagent on an automated cobas c501 and utilizing a commercially available 3-point calibration from Randox instead of a k-factor. **Methods:** The following performance characteristics were evaluated: linearity, imprecision (inter and intra-assay), sensitivity, interferences, method comparison, carryover and stability. A reference interval was verified. **Results:** A patient sample pool with an assigned value from an external laboratory was used for linearity, confirmed from 1.1 to 28 U/L with recoveries ranging from 99.1% to 114.9%. A 1:5 onboard dilution and manual 1:20 dilutions using saline showed acceptable recoveries. The correlation coefficient (CV) at the low limit of quantitation was <15%. Imprecision using serum patient pools ran in triplicate for 5 days at concentrations of 5.0 U/L, 13.8 U/L and 22.6 U/L had within-run CVs of 5.9%, 1.9% and 1.0%, between-run CVs of 0.0%, 0.9% and 0.0%, and overall CVs of 6.1%, 2.4% and 1.9%, respectively. No significant interference was observed up to a hemolysis ≤28 mg/dL, icteria ≤39 mg/dL and lipemia ≤177 mg/dL. Accuracy was assessed by comparing results from 50 serum patient specimens across the linear range with the Roche assay on a cobas c702 (Roche) using the κ-factor at a reference laboratory, resulting on a slope of 0.931, intercept of +0.08, correlation coefficient of 0.9840, overall bias of -0.44 (-5.86%). No carryover was observed up to 250 U/L. Refrigerated and frozen sample stability was adopted from the instructions for use, and we validated aldolase stability up to 96 hours at ambient temperature, which had <15% bias from baseline. The reference interval of 1.5-8.1U/L was verified in 20 apparently

healthy adults. **Conclusion:** The Roche Diagnostics enzymatic assay automation on the Roche cobas c501 and modified for the use of calibrators offered reliable quantitation for aldolase comparable to the use of the same reagent and a k-factor.

A-111

High-Throughput Citrate Quantification by NMR in the Clinical Laboratory

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Background: While urinary citrate is commonly used as a risk factor in the formation of kidney stone, serum citrate is scarcely utilized for disease diagnosis. Association between circulating citrate and all-cause mortality has been reported [1, 2]. The goals of this study were to: (1) develop and evaluate the performance of a new NMR-based citrate assay, and (2) explore the potential association of citrate and mortality in a large prospective multi-ethnic cohort. **Methods:** A high-throughput (~1 minute per sample) nuclear magnetic resonance spectroscopy (NMR)-based method for quantifying serum/plasma citrate was developed and its analytical performance was assessed. To determine if the assay has adequate sensitivity to measure clinically relevant concentrations of citrate, the assay was used to quantify citrate in apparently healthy adults and in a large multi-ethnic cohort. Cox proportional hazards regression analysis (SAS 9.4, Cary NC) was employed to evaluate the association of citrate and 5-year mortality in 5,701 participants in the Multi-Ethnic Study of Atherosclerosis (MESA) study (mean age 62.3 ± 10.3 years). **Results:** The LOB, LOD and LOQ for the citrate assay were 26.0, 39.6 and 77.0 µM, respectively. Linearity was demonstrated over a wide range of citrate concentrations (43.7 to 760 µM). Coefficients of variation (%CV) for intra- and inter-assay precision ranged from 5.2-9.3 and 5.2-9.6%, respectively. Specimen type comparison revealed <1% bias between serum and plasma samples. The reference interval, in a cohort of fasting and non-fasting apparently healthy adult participants (n=567), was determined to be 57.8 to 152 µM with a mean of 98.4 ± 24.5 µM. In the MESA study (n=5,701), higher circulating citrate was associated with older age in men but not in women. During a 5-year follow up, 208 deaths were ascertained. Cox proportional hazards regression models revealed that a 1SD increase in citrate was associated with mortality in MESA participants. The hazard ratio (HR) for the crude model was 1.45 (95% CI: 1.28-1.63, P <0.0001). The association remained significant even after adjusting for age, gender, smoking status and diabetes at baseline. The HR for the fully adjusted model was 1.22 (95% CI: 1.06-1.40, P=0.0048). When performing the analysis by gender, however, citrate remained

significantly associated with mortality in men but not women after adjustment for age and other risk factors. **Conclusions:** The newly developed NMR-based citrate assay exhibits performance characteristics consistent with its use in the clinical laboratory. Citrate levels may be useful for assessing mortality risk in men, in combination with other risk factors.**References**1.Fischer, K., et al., *Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons.* PLoS Med, 2014. **11**(2): p. e1001606.2.Stryeck, S., et al., *Serum Concentrations of Citrate, Tyrosine, 2- and 3- Hydroxybutyrate are Associated with Increased 3-Month Mortality in Acute Heart Failure Patients.* Scientific Reports, 2019. **9**(1): p. 6743.

A-113

A Worldwide Review of Pre-Analytical Errors in Blood Gas Testing

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Introduction: It is well documented that the pre-analytical phase can contribute to up to 75% of errors in laboratory testing.[1] Many laboratories routinely track pre-analytical errors as a part of quality improvement initiatives. In point of care (POC) blood gas testing these metrics are often not available or discussed in the literature. POC/blood gas testing has challenges not found in routine laboratory testing. POC operators may have varying levels of expertise with performing testing as they prioritize patient care, contributing to errors with impact on patient results.[2] To help mitigate pre-analytical factors, the GEM® Premier™ 5000 analyzer with iQM®2 (Intelligent Quality Management 2) (IL, Bedford, MA) offers detection of errors before, during and after sample measurement. This evaluation utilizes iQM2 data to offer new insights into the prevalence of errors in blood gas testing detected during (transient and CO-Oximetry) and after (micro-clots, interferences) samples. **Materials and Methods:** Data from 1,887 PAKs from 650 analyzers in clinical use were reviewed and analyzed for pre-analytical errors detected by iQM2 on 233,530 patient samples tested in multiple settings. Data was analyzed by geographical areas to determine possible differences in pre-analytical factors at different locations. **Results:** Pre-analytical sources of errors were detected with the following prevalence:

Preanalytical category	Error detected by iQM2	Prevalence (%)		
		North America	Europe	Asia
Improper mixing/ anticoagulant	Micro-clots	0.48%	0.99%	0.60%
Inadequate sample preparation and/or patient-specific treatment	Benzalkonium chloride	0.01%	0.04%	0.04%
	Thiopental	0.00%	0.02%	0.04%
	CO-Ox interferences	0.14%	0.19%	0.29%
Transient errors	IntraSpect[1]	0.45%	0.94%	0.56%
Total		1.08%	2.18%	1.53%

1-IntraSpect is an iQM2 quality process that detects transient errors during the sample reading process

Conclusions: Based on the global data analysis, pre-analytical errors in blood gas testing can impact around 1-2 in every 100 patients. The ability to routinely detect, correct and document such errors in blood gas testing offers a unique opportunity to ensure patient safety. Differences observed between geographical areas, especially on micro-clots and transient/sample-specific errors, indicate possible differences in pre-analytical factors that contribute to higher error detection rates. **References:** 1.Bonini P, *et al*. Errors in laboratory medicine. *ClinChem.* 2002;48(5):691-8. 2.D'Orazio,P. Effects of Blood Clots on Measurements of pH and Blood Gases in Critical Care Analyzers.*Point of Care: Journal of Near Patient Testing and Technology.* 2011;10(4)186-8.

A-114

Effect of Ionized Calcium Sample Collection in Unbalanced Heparin Anticoagulant Containers in a Large Academic Hospital

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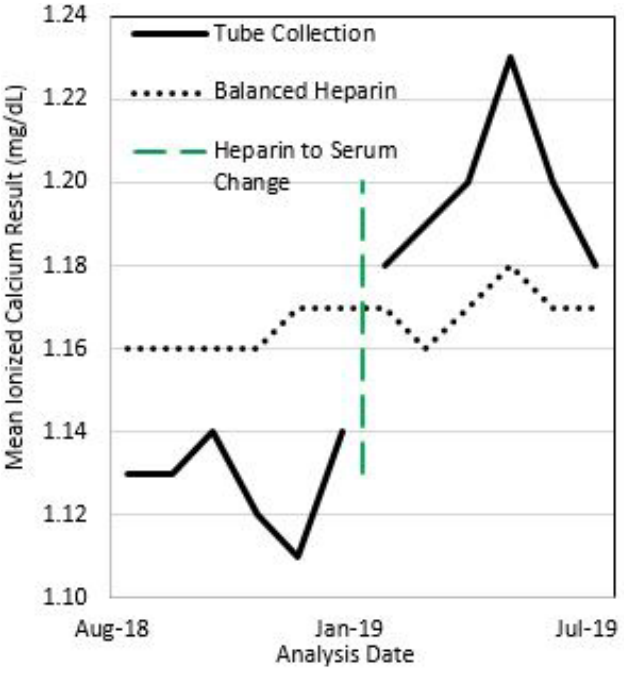
Background: Numerous pre-analytic factors are known to affect ionized calcium (ICa) result accuracy in routine measurements, in particular the anticoagulant type. Lithium and sodium heparin are common anticoagulants utilized for routine chemistry testing, but have calcium binding properties which erroneously decrease the measured ICa from patient samples. In acute care testing, specimen collection syringes are typically coated with a calcium-balanced heparin mixture, which diminishes the effects of heparin binding to the ICa in blood. To mitigate the interference of the calcium-heparin binding, we converted from a sodium heparin anticoagulant to serum. A retrospective analysis was performed to determine the magnitude of the heparin-calcium binding effect.**Method:** Data was collected from inpatients for ±6 months after converting collection containers from a sodium-heparin tube (75 IUP) to a serum separator tube. ICa measurements were performed on commercial blood gas analyzers using ion selective electrodes (ABL825, Radiometer Medical ApS). This data was also compared to ICa results from whole blood electrolyte testing, whose samples are collected in calcium-balanced heparin syringes. **Results:** Heparinized inpatient samples yielded a mean ICa result of 1.13 mmol/L (median=1.13 mmol/L, n=3708) while results from serum testing had a mean of 1.20 mmol/L (median = 1.20 mmol/L, n= 3284). The average observed decrease in results summates to 0.07 mmol/L or 6.0%. A desirable specification for allowable total error is 2.0% (Westgard Quality Requirements) and the theoretical decrease in ICa due to heparin-calcium binding is 0.05 mmoL/L (CLSI C31-A2). Acute care testing of the same inpatient population using calcium-balanced heparin before and after anticoagulant change yielded mean ICa results of 1.16 mmol/L (median=1.16 mmol/L, n=159151) and 1.17 mmol/L (median=1.17 mmol/L, n=152955) respectively (Figure 1). **Conclusion:** This study demonstrates a real-world example of ICa reduction due to sub-optimal sample collection. The observed reduction was greater than the theoretical prediction possibly due to under-filled tubes.



A-116

High-Dose Vitamin C Therapy: A Cause for Concern with Routine Clinical Testing

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Background: Clinicians have been investigating high-dose vitamin C (ascorbate) as a treatment for various illnesses, including sepsis. Blood ascorbate concentrations typically exceed 60 mg/dL but are highly-variable and dependent upon the time of collection since administration, renal function and clearance, as well as, sample handling due to ascorbate instability in biological samples. Ascorbate has been shown to result in falsely elevated point-of-care glucose results. However, the effects on emergent and routine chemistry testing have not been demonstrated previously.

Method: We evaluated the interference of ascorbate on the Abbott POC iSTAT creatinine, Radiometer ABL800 Flex blood gas, Abbott Architect c8000 chemistry, and Roche Cobas 8000 chemistry and immunoassay systems. Following CLSI EP07 guidelines, we investigated the effects in three independent pooled plasma samples spiked with 100 and 400 mg/dL ascorbate, measured in triplicate. Single normal donor whole blood and plasma specimens were also spiked at increasing concentrations of 3.1 - 400 mg/dL ascorbate.Percent and absolute differences of the spiked samples were calculated as compared to control samples. A change in the measured analyte concentration that exceeded the acceptability criteria established for inter-assay cross-checks were considered significant.

Results: The significant interferences resulting from the presence of ascorbate are summarized as the following for initial spiking plasma pools on the Architect c8000: Cholesterol: -189% (at 100 mg/dL ascorbate), Creatinine (enzymatic): -24% (400 mg/dL), Lipase: -20% (100 mg/dL), Triglyceride: -171% (100 mg/dL).

With single donor samples and spiking ascorbate at concentrations ranging from 3.1-400 mg/dL, the following interferences were observed on the ABL800 and Cobas 8000 systems: pH: > -0.05 (50 mg/dL), pCO2: > 9 mmHg (100 mg/dL), HCO3: > -2% (3.1 mg/dL), Base (Deficit/Excess): > -3.3 mmol/L (50 mg/dL), Alkaline Phosphatase: > -8 U/L (200 mg/dL), Bicarbonate: > -29% (200 mg/dL), Bilirubin, Total: > -34% (200 mg/dL), Cholesterol, Total: ERROR: limit low (100 mg/dL), Chloride: > -6 mmol/L (100 mg/dL), Creatinine (Jaffe): > 50% (200 mg/dL), Creatinine Kinase: > -8 U/L (100 mg/dL), Lactate: > -0.5 (100 mg/dL), Triglycerides: ERROR: limit low (100 mg/dL), Uric Acid: > -3.0 (200 mg/dL)

Conclusions: Considering the critical care of septic patients, the effects of ascorbate on point-of-care and blood gas instruments is a cause for concern. Although the majority of Abbott Architect c8000 and Roche Cobas 8000 assays were not affected by high concentrations of ascorbate, significant interference was observed in some tests. Precipitation was observed in the 400 mg/dL spiked plasma pool and further testing is necessary to determine the potential limitations of this interference testing. Further investigation is also underway to more closely test the degree of interference observed in samples collected from patients receiving high-dose Vitamin C infusions.

A-117

Strontium Interference in Clinical Calcium Assays

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Background: In the past two decades, strontium (Sr) has been investigated as an osteoporosis treatment due to its reported anabolic effects on bone. The prescription version of strontium ranelate called Osseor™, was approved for use in Europe, China, and several other countries in 2004. Production of Osseor™ ceased in 2017 due to poor sales and increasing reports of adverse drug events. Various strontium formulations are presently available for consumer purchase at health-food stores and on the internet. Recently, a patient presented with apparent hypercalcemia who had been self-medicating with strontium supplements. **Method:** To determine if the elevated calcium was physiological or an artifact of strontium interference in the arsenazo-dye mediated total calcium (tCa)assay performed in the laboratory, we performed an interference study using three independent pools of remnant patient specimens fortified with 0, 0.03, 0.3, 1.0, 5.0, 12.0, 20.0, 30.0, or 50.0 mcg/ml strontium on an Abbott Architect chemistry c8000 analyzer. The mean of three replicates for spiked samples was compared to control samples. A 0.4 mg/dL difference in tCa for spiked samples vs. control was used as the threshold of significance. To test for interference using ion-selective electrode measurements of ionized calcium(iCa), three replicates of three whole-blood patient pools containing 0, 5.0, and 12.0 mcg/ml strontium were analyzed on a NOVA pHOX Ultra blood gas analyzer. Additional iCa testing at 0 and 50.0 mcg/mL strontium in plasma was performed in duplicate on the NOVA and a GEM5000 analyzers. To assess the possibility of a patient achieving these concentrations of plasma or serum strontium, an ICP-MS strontium method was developed and validated to accurately quantitate the concentration of strontium present in commonly available supplements, some of which listed milligram quantities of strontium on the product label. Supplements were weighed, hydrolyzed, and diluted for ICP-MS strontium analysis according to strontium content listed on the product label. **Results:** tCa measured in strontium-fortified plasma specimens was significantly higher than spiked controls as measured on the C8000. tCa measured in the 3 control pools was 7.98, 7.81 and 7.63, while tCa measured in 12 mcg/mL strontium spiked pools was 12.36, 12.17, and 11.98. The measurement of iCa on NOVA and GEM500 blood gas analyzers proved much less prone to strontium interference. The ionized calcium results from the NOVA and GEM5000 analyzer were slightly increased (14% and 20% respectively) with 50mcg/ml strontium, but still well with in the reference interval of 4.5-5.6 mg/dL. The determined Sr content in four OTC supplements were as follows (*theoretical* concentration, *measured* concentration, percent difference of measured concentration): Life Extension (250mg, 415mg, 49.7%); Solaray (250mg, 244mg, -2.3%); and Best Naturals (340mg, 406mg, 17.7%). **Conclusions:** Arsenazo-dye mediated tCa assays are subject to positive interference by strontium. Patients taking OTC strontium containing supplements can achieve strontium concentrations which have been demonstrated to positively interfere with arsenazo-dye mediated tCa assays. These patients are at risk for apparent pseudohypercalcemia when samples are tested by routine chemistry analyzers using this method. Measurement of ionized calcium may provide a more accurate evaluation of calcemic status in the presence of strontium.

A-119

Retrospective Study of Urianalysis at the Emergency Department of Fundación Santa Fe De Bogota - Evaluation of Pertinence to Offer Clinical Value

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Background: Urinary tract infections (UTI) are the second most frequent infections in the hospital and ambulatory settings. Uroanalysis workup includes urinalysis. As a quality control, we wanted to review the automatized urinalysis requested from the emergency department and relate them with the clinical variables to determine the clinical relevance of urinalysis. The objective of this study was to correlate clinical

characteristics of the adult patients who obtained a subjunctive urinalysis of urinary tract infection in the emergency department at the *Fundación Santa Fe de Bogotá (FSFB)* during the first quarter of 2019.

Methods: A retrospective analysis of 100 automatized urinalysis- requested by the emergency department of the FSFB during the first quarter of 2019 was done. Automated urinalysis were performed by the iQ200 and Aution Max AX-4030 (Beckman Coulter). Urine cultures were performed with the HB&L analyzer from Alifax. The following urinalysis parameters were recorded: proteins, ketones, density, blood, appearance, bilirubins, color, leukocytes, nitrites, pH and sediment. The following clinical data were recorded: Age, gender, temperature, abdominal pain, dysuria, polyakiuria, urinary urgency, vesical tenesmus, hematuria, evolution time, waiting time, history of recurrent UTI, bladder catheter user, antibiotic treatment in the last 90 days, related gynecological condition, surgical treatment, destiny of the patient. Statistical analysis of absolute and relative frequencies, measures of central tendency and dispersion was carried out as appropriate.

Results: 96 patients and 100 urine samples were analyzed. The mean age was 41 years old (range 19-65), 68% were women. Among the 100 samples, 2 patients had proteinuria of 300 mg/dL or more, nitrites were positive in 7 samples, 9 had uncountable erythrocytes and 9 showed presence of bacteria. Gram stain was made in 65 patients, showing 1 type of bacteria in 26 cases (45%), 2 types in 5 cases (7,7%) and 3 types in 3 cases (4.6%). Urine culture was runed in 13 patients of which 10 were positive. From these positive cultures, six were Escherichia Coli and two were Klebsiella Pneumoniae. The main clinical manifestations found were abdominal pain (68%), dysuria (40%) and increase in urinary frequency (30%). 9% of the patients had recurrent urinary tract infections, 14% had received antibiotics in the past 90 days, 10.29% of the women had a related gynecological condition and 76% of the patients received ambulatory care. 16 patients had no UTI symptoms, but urinalysis was taken due to conditions such as nephrolithiasis, immunodeficiency or cancer history.

Conclusion: Uroanalysis in the ED is considered the gold standard for UTI diagnosis. We found a total of 40 patients with UTI symptoms (dysuria), a total of 10 cases had bacteriologic confirmation by culture. 6 out of 10 were nitrites negative, this false negative finding can be explained by short urine retention time. Emphasizing clinical indications and analyzing other laboratory characteristics may improving practice, may reduce the costs and may decrease misdiagnosis. Further studies are granted with a larger number of samples.

A-120

Improved Preanalytic Sample Handling for Whole Blood Thiamine Diphosphate Analysis

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Background: The principal biologically active form of vitamin B1 is thiamine diphosphate (TDP), whose distribution in blood is mainly in red blood cells and much smaller levels in plasma. TDP has been considered the best indicator of vitamin B1 insufficiency. The usual practice in TDP analysis is to freeze the blood specimen immediately and protect it from light until analysis. This restrictive handling causes samples to be rejected and patient care to be delayed. We sought to evaluate specific stability and photosensitivity issues in clinical TDP analysis. **Method**: Plasma samples from 5 healthy donors were obtained in ETDA lavender top tubes, then pooled. One-half of the pooled plasma was used to represent physiologic TDP levels; the other half was spiked with TDP to increase the TDP value by approximately 100 nnol/L. Both the physiologic and spiked pools were then aliquotted for analysis under predetermined time points and conditions. Ambient storage was tested at 4, 8 and 24 hours. Refrigerated (2-8°C) storage was tested at 4, 8, 24, 72 hours and 7 days. Frozen (<-14°C) storage was tested at 3, 8 and 60 days. No specimens were protected from light with amber tubes or foil wraps, although specimens stored in refrigerators or freezers were not exposed to light while in storage. Samples at each time point for each storage condition were assayed in triplicate. 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 for ambient showed values changed <20% for pools tested at 8 hours, but failed at 24 hours. All refrigerated stability pools passed with value changes <20% throughout the 7 days tested. Frozen stability showed acceptable value changes throughout the 60 days tested. **Conclusion:** EDTA Lavender tube specimens have been shown to have acceptable post collection stability for ambient storage up to 8 hours, refrigerated storage up to 7 days and frozen storage up to 60 days for TDP. The study also determined that a need for protection from light with amber tubes or foil wraps was not required. Extending stability and removing the need for light protection will decrease rejected specimens and allow for better patient care.

A-121

Evaluation of Precision Analytical Performance and Bias Estimation of Hepatic Function Assays on the Dimension RXL Max Integrated Chemistry Analyzer

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Background: Liver function tests provide a useful tool to evaluate the hepatocellular injuries and cholestatic pattern and synthetic function of the liver. Accurate and precise assays must be implemented and CLSI EP15-A3 provides a simple experimental approach for clinical laboratories with limited resources to estimate a method's imprecision and relative bias. The objective of this study was to verify the precision analytical performance and bias estimation of hepatic function assays on the Dimension RXL Max Analyzer and and determine if the assays are in accordance to the manufacturer or within other analytical specifications. **Methods**: Hepatic function assays (Alanine aminotransferase-ALT; Aspartate aminotransferase - AST; Alkaline Phosphatase - ALP; Gamma-glutamyl transpeptidase - GGTP; Total bilirubin - TB and Direct bilirubin - DB) were performed on the Siemens Dimension RXL Max Integrated Chemistry Analyzer (Siemens Healthineers Headquarters, Erlangen, Germany) at the Clinical Laboratory Service Navy Medical Center (CEMENA) Callao, Peru. Two levels (L1 and L2) of assayed human based serum chemistry quality control material ACUSERA from Randox (United Kingdom) were used. Each QC level was measured following the CLSI protocol EP15-A3 as follow: five replicates per run, one run per day and during 5 days. Imprecision estimates (IE) for laboratory precision performance of repeatability S_R (%CV_R) and within-laboratory imprecision S_{WL} (%CV_{WL}) were calculated by one-way analysis of variance using Microsoft Excel Analyse-it software. Estimation of relative bias was calculated using RIQAS an ISO/IEC 17043:2010 accredited External Quality Assessment (EQA) scheme based on the mean of laboratory peer group comparison reports from Randox. Estimated imprecision and relative bias was compared to manufacturer specifications claims, CLIA88 specifications and desirable imprecision and bias specifications based on biological variation (BV). **Results**: Overall most of the precision studies agreed with the manufacturer's claims. The observed %CV_R of ALT, AST, ALP, GGTP, TB and DB for L_1, and L_2 were 2.3%, 1.3%; 1.9%, 0.8%; 1.1%, 0.7%; 1,2%, 0.8%; 1.3%, 1.2% and 1.6%, 1.7% respectively. The %CV_{WL} were 2.9%, 2.6%; 3.8%, 2.1%; 3.5%, 2.0%; 2.9%, 2.8% and 2.4%, 2.3%. The %CV_R (1.1%) and %CV_{WL} (3.5%) of ALP for L_1 (mean 157 U/L), %CV_R (1.7%) and %CV_{WL} (2.3%) of DB for L_2 (mean 4.3 mg/dL) and %CV_R (1.3%) of ALT for L_2 (mean 169 U/L) exceeded the MSC and were within the CLIA 88 and/or desirable BV specifications. The L_1, and L_2 observed mean were 48 and 142 U/L for ALT, 51 and 161 U/L for AST, 155 and 324 U/L for ALP, 68 and 198 U/L for GGTP, 1.63 and 4.8 mg/dL for TB and 0.749 and 1.01 mg/dL for DB. Analytical relative %bias estimation of ALT, AST, ALP, GGTP, TB and DB for L_1, and L_2 were -11%, -4.6%; 4.9%, 3.3%; -6%,-4.8%; -4.5%, -1.3%; 0.2%,1.8% and 4.7%, 1.9%, respectively and all were within desirable imprecision and bias specifications based on biological variation (BV). **Conclusion**: The hepatic function assays on the Dimension RXL Max Integrated Chemistry Analyzer demonstrated good precision with an acceptable relative bias agreement and consistency with the manufacturer specifications claims and/or desirable imprecision and bias specifications based on biological variation.

A-122

Correlation between Albumin and Sodium in CA125-and-Gender-Based Subsets of Maintenance Hemodialysis Patients

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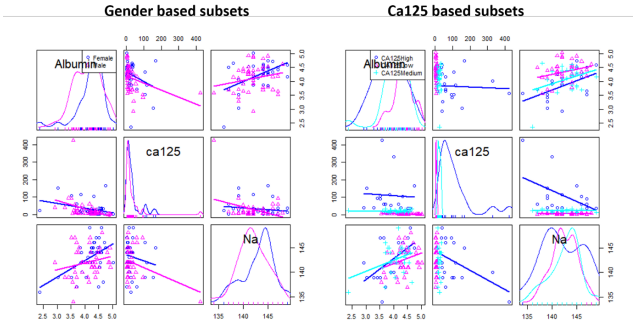
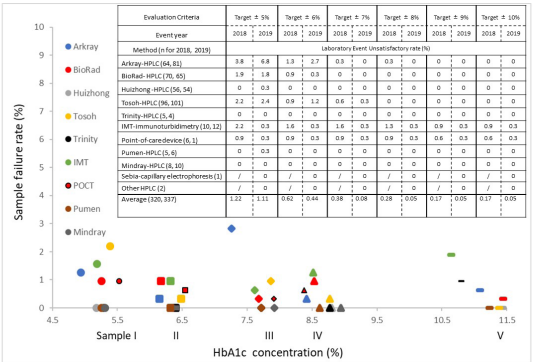
Background: CA125, classically known as an ovarian cancer marker, has of late, found multiple other applications including its usefulness as an indirect marker of heart failure. Serum albumin is an important prognostic indicator of health status in maintenance hemodialysis patients that has many implications beyond nutrition. Serum albumin and sodium are also classically related to the blood volume. **Method:** Correlation between serum albumin and sodium was explored in 500 routine samples from maintenance hemodialysis patients in a tertiary care center in Eastern India. Serum CA125 was measured in 100 of these patients on Beckman-Coulter-Access-2 with IRB approval. **Results:** Albumin was found to have weak but highly signifi-

cant positive correlation with sodium (r=0.20, $p < 0.00001$). Albumin had significant moderate negative correlation with CA125 (r=-0.46, $p < 0.001$). The distribution of CA125 was "log-normal", mean 40U/ml, median 12U/ml, Shapiro-Wilk $p < 2.2*10^{-16}$ transformed to 0.06 on log. Based on standardized 35U/ml cut off, 25% of patients classified as "high-CA125" group. The median (12U/ml) was used as another intermediate cut off and the population was divided into 3 subgroups (high, medium & low). The albumin to sodium relation was found to be correlated with progressively increasing slope & correlation in the 3 groups, of which however, the "high-CA125" group showed significant p value. Male (n=296) and Female (n=190) subgroups showed positive correlations between albumin and sodium independently of CA125; Males showed weaker correlation whereas females showed better correlation than the overall: r= 0.35 in Female, 0.12 in Male and 0.20 overall, $p < 10^{-5}$ each for Females and overall, 0.047 for Male). **Conclusion:** Female-gender and high-CA125 subsets independently show better correlation between albumin and sodium than overall. The progressively increasing slope of the 3 regression lines for CA125 based subsets is likely because CA125 has association with volume status in hemodialysis patients.





Evaluation Criteria	Target ± 5%		Target ± 6%		Target ± 7%		Target ± 8%		Target ± 9%		Target ± 10%	
Event year	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Method (n for 2018, 2019)	Laboratory Event Unsatisfactory rate (%)											
Arkray-HPLC (64, 81)	3.8	6.8	1.3	2.7	0.3	0	0.3	0	0	0	0	0
BioRad- HPLC (70, 65)	1.9	1.8	0.9	0.3	0	0	0	0	0	0	0	0
Huizhong -HPLC (56, 54)	0	0.3	0	0	0	0	0	0	0	0	0	0
Tosoh-HPLC (96, 101)	2.2	2.4	0.9	1.2	0.6	0.3	0	0	0	0	0	0
Trinity-HPLC (5, 4)	0	0	0	0	0	0	0	0	0	0	0	0
IMT-immunoturbidimetry (10, 12)	2.2	0.3	1.6	0.3	1.6	0.3	1.3	0.3	0.9	0.3	0.9	0.3
Point-of-caredevice (6, 1)	0.9	0.3	0.9	0.3	0.9	0.3	0.9	0.3	0.6	0.3	0.6	0.3
Pumen-HPLC (5, 6)	0	0.3	0	0	0	0	0	0	0	0	0	0
Mindray-HPLC (8, 10)	0	0	0	0	0	0	0	0	0	0	0	0
Sebia-capillary electrophoresis (1)	/	0	/	0	/	0	/	0	/	0	/	0
Other HPLC (2)	/	0	/	0	/	0	/	0	/	0	/	0
Average (320, 337)	1.22	1.11	0.62	0.44	0.38	0.08	0.28	0.05	0.17	0.05	0.17	0.05

A-125

CLIA (2019 Proposed) Proficiency Testing Criteria for HbA1c - 5% vs 10% or in-between?

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Background: 2019-proposed revisions to CLIA '88 proficiency testing (PT) regulations recommended adding hemoglobin A1c (HbA1c) with an acceptance limit (AL) of 10%. [Federal Register V84(23), 4 February 2019]. This limit is more lenient than that currently used by other PT programs and has aroused a controversy. **Objective:** To estimate impact of various ALs on HbA1c-PT. **Methods:** Ten PT samples were prepared from discarded patient's EDTA-whole blood specimens, frozen and distributed (0.5 mL/sample) in 2018 and 2019 PT events, to 320 and 337 participant laboratories, respectively, in Shanghai. Participant laboratories measured HbA1c concentrations with their respective methods (Table). SCCL established target values using 4 IFCC-certified reference measurement procedures, two ion-exchange high-performance liquid chromatography (HPLC), one boronate-affinity-HPLC and one capillary-electrophoresis. The target value was derived from the mean of results of duplicate analysis using the reference-methods. Participant results were graded using the target ± AL (5%, 6%, 7%, 8%, 9% and 10%). A result outside the target ± AL was considered a Failure Result. We calculated sample failure rates, i.e., percentage of Failure Results of total sample results (Figure for ±5%, 2018 event). We assigned a Laboratory Event Unsatisfactory Status when Failure Results of ≥2 out of 5 samples per PT event (Table). Laboratory Event Unsatisfactory rates (%) were calculated, per peer group, i.e., number of unsatisfactory per event divided by total number of laboratories for the peer group of that event. **Results:** The targets of the PT samples were 5.2%, 6.4%, 7.7%, 8.7% and 11.5% for 2018 event, and 5.2%, 6.1%, 6.9%, 7.7% and 9.9% for 2019 event. The Laboratory Unsatisfactory Event rates showed (Table) that when using the target ± AL ≥6%, Laboratory Event Unsatisfactory rates were all under 5%. **Conclusion:** Using commutable samples with the targets set by SCCL, simulation analysis indicates an AL for HbA1c PT of ± 6% be acceptable, even at low concentrations.

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Endocrinology

A-127

Manual Calculation of Hemoglobin A1c% using Sebia Capillarys2 Chromatogram Data for an Apparent Compound Heterozygote for Hemoglobin Delta Chain Variants (Absence of Normal A2)

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BACKGROUND: Measurement of hemoglobin A1c% at our institution is conducted using capillary electrophoresis (CE, Sebia Capillarys2). We received a sample having an uncommon CE pattern without an identifiable A2 peak. Identification of an A2 is one criterion used by the instrument for validity of the CE chromatogram. In the absence of an A2 peak, the instrument will not automatically calculate the A1c%. Because of the unusual nature of this case, we report on our investigation of the sample and its A1c% measurement outcome. **METHODS**: Measurement of A1c by capillary electrophoresis (CE) for normal subjects involves automated identification of peaks for A1c, other A, A0, and A2. A1c is calculated automatically from the ratio R = A1c/(A1c+A0) according to a linear calibration curve: A1c% = mR + b. In the absence of an A2 peak, the instrument will not automatically calculate A1c%. The sample was therefore analyzed for A1c% by boronate affinity HPLC (Trinity Biotech Ultra2) as an alternative method. The result was compared to that obtained by manual calculation of A1c% from R obtained from the CE chromatogram using the calibration curve. The absence of A2 in CE was in the presence of two unidentified small peaks, suggesting that the patient might be a compound heterozygote for hemoglobin delta chains. The sample was therefore analyzed for hemoglobin variants using ion-exchange HPLC (Trinity Biotech Ultra2). **RESULTS**: Hemoglobin variant analysis by HPLC showed a pattern consistent with the presence of a delta chain variant with an elution time of A2' or similar to A2' (e.g., A2 Grovetown). In consideration of the two apparent A2 variant peaks in CE, HPLC results were also consistent with presence of a second delta chain variant sharing elution time with hemoglobin A (e.g., A2 Fitzroy). Boronate affinity HPLC gave A1c = 7.9%. In comparison, manual calculation of A1c% from the CE chromatogram gave an essentially identical result: A1c = 8.0% (R = 0.086; A1c% = 73.0 R + 1.71). The sum of variant A2 peaks for this patient (2.2%) was in the 54%-ile of the distribution of A2 results for patients on the same CE run having normal CE patterns (A2 = 2.2 ± 0.24 %; n = 50), consistent with the compound heterozygosity for delta chain variants in this patient. **CONCLUSIONS**: CE and HPLC analysis of hemoglobins for this patient were consistent with compound heterozygosity for delta chain variants, leading to a complete absence of a normal A2 peak in CE chromatograms. This circumstance precludes automated calculation of A1c% using the Capillarys2 system. However, manual calculation of A1c% for this sample yielded the correct A1C% according to alternative HPLC analysis. Although this circumstance is likely to be rare, manual calculation in such cases makes unnecessary the additional cost and delay in reporting of A1c% measurement by an alternative assay.

A-129

Thyroid Testing Ordered per Patient Visit along One Year: Clinicians Don´t Follow Guidelines.

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Background There is a strong growing global interest in promoting high-value care initiatives, including laboratory testing for diagnosis with multiple professional society's guidelines, governmental campaigns and Choosing Wisely recommendations. Inappropriate laboratory testing is prevalent, with much of it in the form of over-utilization but also repetitive testing. Thyroid function evaluation is very common because thyroid diseases are highly worldwide prevalent in all ages. Besides, symptoms of thyroid diseases are miscellaneous and even on-treatment patients can present unspecific symptoms that thyroid function must be checked for an appropriate management. Given the large volume of thyroid function testing and afterwards targeting policy changes that would have more impact on laboratory utilization, we proposed to evaluate the ordering prevalence of each thyroid test and also the ordering practices in our laboratory during 12 months. **Methods** The study was carry out in the central region of Brazil, including only outpatient laboratory tests. Pregnant women and samples from Hospital locations were excluded from analysis. The number of patient laboratory visits and physician´s orders were obtained by reports from our laboratory´s registration systems. Thyroid function, including TSH, total and free T4 (TT4, FT4), total and free T3 (TT3, FT3) and reverse T3 was retrieved from the laboratory information system. Thyroid autoimmunity, including thyroperoxidase, thyroglobulin and TSH receptor antibodies (TPOAb, TgAb, TRAb) were also analyzed. All thyroid function and antibodies tests were performed in the same platform, using commercial kits (Roche, Cobas 8000). Normal reference range were determined according to age and manufacturer's information. Test results falling inside the reference range were termed "normal result". **Results** From January to December 2018, a total of 162,409 patients performed 537,415 thyroid tests, from one (only TSH 99.3%) to 9 tests, median 3 tests per patient. In all ages, TSH+FT4 was the most common thyroid function order (53.5%), followed by only TSH (12.8%). Next combination TSH+FT4+TT3 was more common in patients >18 years-old followed by TSH+FT4+TPOAb+TgAb. Of the 129,076 patients with all normal thyroid tests, 22,906 patients (17.7%) repeated their thyroid tests during the study period, mainly TSH+FT4 and 6,014 patients (26.2%) repeated for the third time, even all being within normal ranges. It is noteworthy that 3,700 patients repeated their normal thyroid function tests in less than 6 weeks interval, with the same physician requests in 13%. As we studied only orders outside Hospitals, we assumed that patients performed thyroid function evaluation for ambulatory diagnose or to control a previous known thyroid disorder. Therefore repetition of thyroid test and in short interval after normal results are not recommended. Normal TSH range are wide and its concentrations are related to FT4, justifying combined order. But TPOAb and TgAb measurements are not necessary to be measure together and even to repeat. **Conclusion** Thyroid function tests are frequently repeated even after results within normal range in our cohort of outpatients. The most common request is TSH+FT4 denoting that clinician do not follow the guidelines to evaluate or reevaluate the patient. Our effort will focus on communication among physicians simultaneously treating ambulatory patients to avoid repetition.

A-132

Factors Affecting Preanalytical Stability of Intact Parathyroid Hormone in Serum and Plasma

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Background: The hallmark of an outstanding clinical laboratory is the consistency of producing reliable results. Intact parathyroid hormone (IPTH) is one of the hormones that can be affected by different factors. In this study, we have investigated IPTH values using different types of tube (Plain, SST and EDTA), the effects of delay in separation and the stability of IPTH at different time intervals using the most common storage conditions used in the clinical laboratories. **Methods:** Blood samples from 30 healthy subjects were collected in three types of tubes plain (serum), SST (serum), and EDTA (plasma). All samples from each type of tubes were run immediately after separation. Then, serum and plasma were aliquoted and stored in room temperature (25°C), fridge (4°C) and freezer (-20°C). All samples were processed at different time days (2, 4 and 8). All plasma and serum samples were delayed in separation for two hours before they have been analyzed using chemiluminescent immunoassay (Architect). Total allowable error of 30% was considered for clinically significance using the Clinical Laboratory Improvement Amendment as a reference. **Results:** Comparing of three groups means (SST serum, plain serum and EDTA plasma) after an immediate run was not significantly different (p= 0.95). By comparing the two means of delayed in separation for plain serum and plasma, they were statistically significant (p<0.001) when compared to zero time. However, the means of percentage changes were 3.77% and 7.37% for serum and plasma respectively. IPTH means in subsequent days by using different storage conditions were statistically significant when compared to the zero-time (p<0.001). However, when changes were compared to total allowable error (30%), stored serum samples at room temperature were significantly different in all days, while plasma was different in day 8 only. Fridge storage condition has no effect on both serum and plasma. Freezer stored IPTH samples were the best in stability for both plasma and serum. **Conclusion:** Freshly collected IPTH samples can be analyzed using EDTA, SST or plain tubes. Samples can be reached to the lab within 2 hours of collection. Plasma IPTH at room temperature has longer stability than serum. Plasma and serum IPTH can be stored and analyzed within 1 week of storage in fridge or freezer.

A-134

LC-MS/MS Measurements of PTHrP Revealed Higher Prevalence of PTHrP caused Hypercalcemia in Men than Women

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Background. Parathyroid hormone related protein (PTHrP) has close homology with parathyroid hormone (PTH). The main clinical utility of PTHrP is to aid in determining the cause of unexplained hypercalcemia, however, much remains unknown about its function. Considering both PTHrP and PTH are involved in Ca homeostasis, we evaluated the association between concentrations of Ca, PTH and PTHrP, and between-sex and age group differences in clinical samples. **Methods.** We recently developed, validated, and implemented a LC-MS/MS method for PTHrP measurement in clinical samples (Clin Chem 2016, 62:218-26). Sample preparation is performed as follows: stable isotope labeled internal standard (^{15}N-labeled PTHrP) is added to the samples, PTHrP is enriched using anti-PTHrP antibody conjugated to magnetic beads, beads are washed, PTHrP is digested with trypsin, and PTHrP-specific peptide is analyzed by LC-MS/MS. We assessed clinical performance of the assay by review of Ca, PTH, and PTHrP concentrations measured in consecutive routine patient samples (n=2,701; 60.5% women and 39.5% men) submitted for testing to a large reference laboratory. Among the analyzed samples, 31.1% were from adults (18-59y), 52.9% from older adults (60-79y) and 16.9% from elderly (>79y). PTHrP was measured using this LC-MS/MS method; Ca and PTH were measured using electrochemiluminescence (Cobas 8000, Roche Diagnostics). The imprecision of all assays was <10%. Reference intervals for PTHrP were <3.3 pmol/L and <2.2 pmol/L in women and men, respectively; reference interval for calcium was 8.4-10.2 mg/dL, and 15-65 pg/mL for PTH. **Results.** Statistically significantly higher PTHrP concentrations were observed in men compared to women (p=0.043). Median (mean) PTHrP concentrations in samples from women and men were 2.4 (6.3) and 2.5 (10.4) pmol/L, respectively. PTHrP concentrations were above the sex-specific reference interval in 39.1% of samples (n=1056). No between-sex or among age group differences were observed in Ca concentrations. A direct relationship was observed between Ca and PTHrP concentrations (ρ=0.189, p<0.0001), while inverse relationships were observed between Ca and PTH (ρ=-0.393, p<0.0001), and PTH and PTHrP concentrations (ρ=-0.226, p<0.0001). Assessment of PTHrP concentration distributions in samples from women (n=472) and men (n=446) with unexplained hypercalcemia (Ca >10.2 mg/dL and PTH< 15 pg/mL) revealed elevated PTHrP concentrations in 35.0% of women (n=165) and 59.4% of men (n=265). Odds ratios for PTHrP as a cause of unexplained hypercalcemia were 0.7 in women and 2.2 in men. Higher PTH concentrations were observed in elderly than in adult (p=0.0070) and older adult (p=0.0064) groups of men; no among age group difference in PTH concentrations was observed in women. Distribution of PTH concentrations in women and men of the same age groups showed higher concentrations in women than in men in the adult (p=0.019) and the older adult (p<0.0001) groups.**Conclusions.** In routine clinical samples and samples from patients with unexplained hypercalcemia, PTHrP concentrations above the reference interval were observed more frequently

in men than women. This data suggests a possible role for PTHrP in hypercalcemic conditions that have a higher prevalence in or are specific to men.

A-135

Establishment of Reference Intervals for Serum Insulin-Like Growth Factor 1 in Healthy Korean Adults

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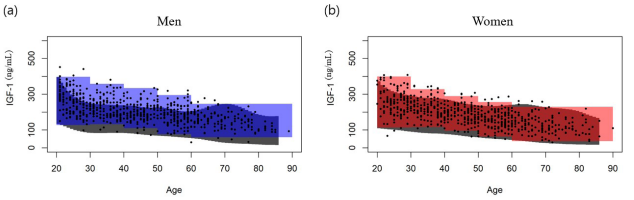
Background: Measurement of serum insulin-like growth factor-1 (IGF-1) is important for diagnosing and monitoring patients with growth hormone (GH)-related diseases, and accurate interpretations necessitate the use of appropriate reference intervals. **Methods:** To establish reference intervals for healthy Korean adults using a chemiluminescence immunoassay (Liaison), at least 120 healthy subjects (N=1358, 688 men and 670 women) selected after applying exclusion criteria were enrolled in each age group (20-29, 30-39, 40-49, 50-59,≥60). Serum IGF-1 was measured by Liaison, and the parametric method was applied to establish reference intervals. Concordance of patient classification based on reference intervals, manufacturer's interval, and standard deviation score (SDS) was evaluated. **Results:** New reference intervals had higher upper and lower limits than those specified by the manufacturer. This difference was particularly greater in case of men <60 years. The agreement between classification using new reference interval and the manufacturer's reference interval was 75.0% (weighted kappa, 0.17) in men and 91.0% (weighted kappa, 0.41) in women. On considering the SDS from a previous study, the agreement was 91.9% (weighted kappa, 0.51) in men and 92.5% (weighted kappa, 0.53) in women. **Conclusion:** As assay method and race may affect IGF-1 levels, reference intervals should be established not only based on age and sex, but also on ethnicity and assay method.



A-141

Interference of Separator Gels and EDTA of Blood Collection Tube in Pediatric Serum Steroid Hormone Measurement using High Performance Liquid Chromatography-Tandem Mass Spectrometry

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BACKGROUND: Measurement of serum steroid hormones is widely used in the diagnosis and following up of various endocrinologic diseases. Especially in pediatric patients who are suspected to have defects in steroidogenesis, their various serum steroid hormones should be measured, though several pediatric steroid hormones show generally low level than those of adult individual due to sexual immaturity, which are vulnerable to interference. Since we generally use serum separator tubes (SST) when we separate blood samples to serum and blood clot, separator gels can affect the concentration of analytes including steroid hormones. We have observed several suspicious cases of separator gel interference in serum steroid hormone measurements from specific SST tubes, so we conducted a pilot study of serum separator gel interference in steroid hormone measurement using high performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS).

METHODS: We collected the remaining blood samples of EDTA tubes from four pediatric individuals less than 6 years old (Three males and one females). These samples were centrifuged and aliquoted to SST BD Vacutainer SST™ (SST I) tubes, then measured each other after they stored in a refrigerator for a day. Since two of four patients also have the remaining samples stored in BD SST™ II Advanced (SST II) tubes and SST I tubes , serum steroids of these samples were also measured. Furthermore, we collected 12 pediatric blood samples (7 Males and 5 females) stored in EDTA and plain tubes to check the interference of EDTA in serum steroid hormone measurements. We measured dehydroepiandrosterone (DHEA), androstenedione, testosterone, progesterone, 17-hydroxyprogesterone, pregnenolone, 17-OH pregnenolone in all samples by HPLC-MS/MS.

RESULTS: In pediatric samples, testosterone (0.827 vs 0.027 ng/dL), 17-hydroxprogesterone (0.23 vs 0.07 ng/dL), pregnenolone (5.92 vs 2.93 ng/dL) and 17-OH pregnenolone (4 vs 0.23 ng/dL) level was higher in the samples of SST I tubes than those of EDTA tubes, and DHEA was unavailable to measure in SST I tube due to interference. Steroid hormones of SST I samples also show higher level than those of SST II tubes in testosterone (4.93 vs 2.66 ng/dL), 17-hydroxyprogesterone (1.04 vs 0.56 ng/dL), 17-OH pregnenolone (14.5 vs 0.15 ng/dL). Steroid hormone levels of EDTA tube samples were not significantly different from those of plain tube samples except for DHEA (P = 0.01) which show higher concentration in EDTA samples (2.00 vs 1.23 ng/dL).

CONCLUSION: In pediatric steroid hormone measurement using HPLC-tandem mass spectrometry, several steroid hormone concentrations from serum samples collected in BD Vacutainer SST™ tubes can be increased due to the interference of separator gels in tubes. Especially serum DHEA concentration is not only unmeasurable in the sample of BD Vacutainer SST™ tube, but also may appear to be increased than that of EDTA tube samples

A-142

Improved Recognition of 25-Hydroxyvitamin D_2 by Two Automated Immunoassays

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Background: While mass spectrometry-based reference methods provide accurate measurement of 25-hydroxyvitamin D (vitamin D), high test volumes often necessitate the use of automated immunoassay measurements. A historic and key limitation of immunoassay methods has been uneven recovery of 25-hydroxyvitamin D_2 (D2), with assays exhibiting over- or under-recovery. This concern is particularly relevant for a patient population in which use of high-dose D2 supplements is common, as under-recovery may result in over-supplementation, while over-recovery could lead to insufficiency if supplementation is consequently withheld. We evaluated two automated immunoassays for their recovery of D2, using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) measurement at a national reference laboratory as a reference method. **Methods:** Forty-nine serum specimens submitted for vitamin D measurement were assayed for total vitamin D by the Roche Elecsys® Vitamin D total II and IDS®-iSYS 25 VitD^S immunoassays and shipped overnight for D2 and 25-hydroxyvitamin D_3 (D3) measurement by LC-MS/MS. Immunoassay vitamin D results were assessed against the LC-MS/MS method for suitability using predefined agreement criteria (the greater of 5 ng/mL or 20% of the LC-MS/MS total vitamin D result). Estimated D2 content was calculated for each specimen by subtracting the LC-MS/MS D3 result from the immunoassay total. Estimated mean immunoassay bias was calculated, and vitamin D status was determined for each specimen according to the 2011 Endocrine Society practice guidelines.

Results: Seven specimens for the Roche assay (14%) and eleven specimens for the IDS assay (22%) exceeded desirable error limits. Mean bias (±SD) for samples with D2 concentrations less than 5 ng/mL were 0.25 ng/mL (±6.30) for Roche and -1.45 ng/mL (±6.82) for IDS. Mean bias (±SD) in samples with D2 concentrations greater than 5 ng/mL was 3.19 ng/mL (±6.61) for Roche and 5.52 ng/mL (±6.36) for IDS. Median estimated D2 recovery was 87.1% (interquartile range 76.0-111.3%) for Roche and 120.6% (interquartile range 105.3-133.4%) for IDS. Vitamin D status was misclassified in seven samples by the Roche assay and three by the IDS assay; however, for all but one specimen, the immunoassay result was within 1.7 ng/mL of the diagnostic cutoff. The remaining specimen had a LC-MS/MS vitamin D of 107 ng/mL, indicating intoxication, and was assigned a value of 91.1 ng/mL by the Roche assay and 88.3 ng/mL by the IDS assay, indicating sufficiency in both cases. **Conclusion:** Both assays exhibit improved recognition of D2 relative to earlier generations. Although both assays exhibit worsening proportional bias with increasing D2 content, in practice, the obtained result is sufficient for appropriate clinical classification of vitamin D status.

A-144

Measurement of Aldosterone Levels: Analytical Performances of a New Fully Automated Chemiluminescent Immunoassay

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Background: Aldosterone is a mineralocorticoid hormone and one of the most important hormonal regulators of salt and water balance. Testing for aldosterone is important for the differential diagnosis of hypertensive subjects when primary aldosteronism is suspected. Reliable measurement of aldosterone is also critical for adrenal vein sampling and the subtyping of primary aldosteronism. Our study objective was to determine the performances of a novel fully automated chemiluminescent assay for testing of aldosterone. **Methods:** We evaluated the Maglumi® (Snibe) aldosterone assay a fully automated competitive immunoluminometric method based on a solid-phase, paramagnetic microbeads, coated with a monoclonal antibody targeting aldosterone epitopes. Assay imprecision was assessed with two pools of plasma samples targeting two different aldosterone concentrations. Method comparison was performed with a chemiluminescent immunoassay (CLIA) (DiaSorin) with 24 patients' samples. **Results:** The between-run coefficients of variation of the Maglumi® aldosterone assay were 12.2 and 4.1 % for concentrations of 120 and 428 pg/mL, respectively. The median aldosterone levels were 192 pg/mL (range: 75 - 763) with the Maglumi assay and 144 pg/mL with the CLIA method (range: 22 - 901). The correlation between the both methods was weak (r=0.65, p<0,05). The Passing-Bablok regression analysis showed a slope of 0.65 and an intercept of 85. Bland-Altman plot evidenced a bias between the methods with a mean bias of 23 pg/mL. **Conclusions:** Our preliminary data showed good imprecision for Maglumi aldosterone automated immunoassay. However, the results of our method comparison showed that aldosterone automated immunoassays are not commutable and that careful validation with definition of specific reference interval remains mandatory before routine use.

A-145

Mitochondrial Function is Minimally Affected during the Development of Non-Alcoholic Fatty Liver in Obese Mouse Models

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Background: Non-alcoholic fatty liver disease (NAFLD), characterized by hepatic steatosis and non-alcoholic steatohepatitis (NASH), is caused by the deregulation of hepatic energy metabolism. However, the association between NAFLD progression and mitochondrial function is debatable. Our objective is to investigate the association between mitochondrial function and NAFLD

Methods: We applied high-resolution respirometry, blue native polyacrylamide gel electrophoresis with in-gel activity, immunoblot analysis, and transcriptome profiling in multiple obesity-induced mouse models that had varying degrees of NAFLD, to identify the association between mitochondrial function and NAFLD.

Results: We found a significant but mild decrease in hepatic mitochondrial respiration in some but not all of the NAFL or NAFLD mice when compared with mice fed with standard diet. Notably, the oxidative damage to mitochondrial protein and activities of mitochondrial oxidative phosphorylation complexes remain unaffected in the liver of mice during the development of NAFLD. Indicating that the hepatic mitochondrial function was not associated with obesity-induced NAFLD progression. Moreover, transcriptome profiling revealed a decreased hepatic mitochondrial function in mice with NASH only, at high histological grade. This suggests that impaired hepatic mitochondrial function was not closely associated with the development of NAFLD. This was confirmed further by the fact that the levels of hepatic mitochondrial transcriptional factors were not altered in mice during the development of NAFLD.
Conclusion:

Our results imply that impaired hepatic mitochondrial function is at least not the driving force of obesity-induced NAFLD, and therapeutic strategies targeted at the mitochondria for the treatment of NAFLD should be reconsidered.

A-146

Fast and Reliable Method of Salivary Cortisol Quantification using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Background: Background: Cortisol is one of the most important glucocorticoids in regulating human metabolism. Midnight salivary cortisol has been shown to correlate well with free cortisol (F-cortisol) concentration in serum and is one of the first tests recommended for the diagnosis of Cushing's syndrome. The purpose of this study was to validate the Cortisol salivary quantification using LC-MS/MS Waters® Xevo-TQS. **Materials and Methods** The procedure described here involves centrifugation of the saliva samples to remove solids and mucus strands before they are diluted with methanol containing carbonate internal standard cortisol-$^{13}C_3$ (IS). The samples are then subjected to separation on an Acquity BEH C18® analytical column (50 x 2.1mm, 1.7µm). Quantification is achieved by comparing the responses of a given sample to the responses of the calibrators of known concentrations. Analytical specificity is ensured by using multiple reaction monitoring with fragment ions that are unique to cortisol and IS, 363.5 >121 and 366.6 >124.3 respectively. The mobile phase was H_2O: Methanol with formic acid 0.1% (88:12-v/v) with a flow rate of 0.3 mL/min. The linearity was observed in the expected concentration range. F-cortisol saliva was used as a biological matrix for the study. **Results**: The linearity was studied using enriched samples of cortisol from 1.0 to 30.0 ng/mL and the saliva samples were evaluated six times, each. The intra-assay and inter-assay precision and accuracy are demonstrated in table 1. The retention time was 0.76 min and the total analysis time was 2.5 min. Linearity was studied in the concentration range from 1.0 to 30.0 ng/mL with a coefficient of determination (R^2) of 0.993639. **Conclusion:** The method was quick and efficient in determining salivary cortisol. The efficiency and selectivity combined with the technical robustness can be employed to cortisol dosage in the control of diagnosis of Cushing's syndrome.Table 1: Results found

Concentration ng/mL	Precision		Accuracy	
	Intra-Assay (RSD%) n=6	Inter-assay (RSD%) n=18	Intra-Assay % n=6	Inter-assay % n=18
0.1	7.70	N.D.	100.0	N.D.
0.2	6.85	N.D	100.0	N.D
0.5	5.74	9.6	94.0	94.0
1.0	6.38	N.D	95.0	N.D
2.0	4.33	N.D	95.0	N.D
5.0	1.23	N.D.	95.2	N.D.
7.5	0.75	N.D	108.5	N.D
10.0	1.85	4.6	98.9	100.2
20.0	0.54	5.6	108.9	103.3
30.0	2.33	N.D	102.3	N.D

N.D. Not determined

A-147

Evaluation of Equilibrium Dialysis ID UPLC/MS/MS Candidate Reference Measurement Procedure for Free Thyroxine in Human Serum

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Background: Accurate and reliable measurement of free thyroxine (FT4) is critical for diagnosis and treatment of thyroid diseases. However, there are concerns about the accuracy and reliability of FT4 measurements in patient care and research. The CDC Clinical Standardization Programs (CDC CSP) collaborate with the American Thyroid Association (ATA), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and Partnership for the Accurate Testing for Hormones (PATH) to address these concerns through the efforts for standardization of clinical laboratory measurements of FT4. **Methods:** As part of the efforts for standardization of clinical FT4 measurements, CDC CSP developed a candidate FT4 reference measurement procedure (cRMP), based on the IFCC RMP developed at Ghent University, using equilibrium dialysis ultra-performance liquid chromatography tandem mass spectrometry (ED-UPLC/MS/MS) for determination of FT4 in human serum. FT4 in serum was separated from protein-bound thyroxine by equilibrium dialysis in Teflon dialysis cells (1 mL). Dialysis reached equilibrium at 37°C after 4 hours dialysis. FT4 in the dialysate was purified by extractions prior to LC-MS/MS analysis. To determine the concentration of FT4 in serum, certified primary reference materials are used to prepare calibration materials. Chromatographic separation was achieved on a C18 reverse phase column with a gradient of methanol and water with 0.1% formic acid. Quantification of FT4 was performed by using selective reaction monitoring in positive electrospray ionization mode. To achieve appropriate precision and accuracy, stable-isotope dilution, bracketed calibration, and gravimetric measurements were used in the FT4 cRMP. **Results:** The within- and between-day imprecision for the CDC RMP are 2.2-3.9% and 1.8-2.6%, respectively. Factors affecting measurement accuracy were also investigated to optimize performance of the method. CDC cRMP was compared with the RMPs at Ghent University and other two independent laboratories. The CDC cRMP reported a bias within ±2.5% of the mean of all laboratories. The sensitivity of the CDC cRMP of FT4 allowed quantification of samples from normal and hypothyroid patients. Our preliminary study also found an average difference of 37.7% between a commercial immunoassay and the CDC cRMP among hypo-, eu-, and hyperthyroid patients. The results indicated the need for standardization of commercial FT4 assays. We are conducting FT4 reference value assignments with CDC cRMP for single-donor serum samples to support upcoming CDC Hormone Standardization (HoSt) program for FT4 assay. These reference materials will be applied to assess the accuracy of the commercial FT4 assays and to help in recalibrating these assays as needed. **Conclusion:** Overall, we established accurate, precise and sensitive higher-order FT4 cRMP that is suitable for standardization of the clinical laboratory measurements of FT4.

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Evaluation of Abbott Fertility and Pregnancy Assay Lot-to-Lot Variability

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Background: The decision to start a family can be an exciting, emotional, and potentially anxious time for individuals and couples. There are many paths to pregnancy and for some women and men this path includes medical assessment for infertility. Multiple hormone assays aid in assessing fertility status, confirming pregnancy and monitoring women throughout pregnancy. Physicians and patients rely on the accuracy and consistency of these test results to help find answers and monitor status.

Objective: The goal of this study was to evaluate the reagent lot-to-lot performance of seven hormone assays (FSH, LH, Progesterone, Prolactin, Estradiol, Testosterone and Beta-hCG) on the Abbott Architect platform in both a manufacturing and hospital clinical laboratory setting.

Methods: Quality controls (QC) with values across the measurement range and human serum panels targeted at or near medical decision points were tested during reagent lot manufacturing. Data represent multiple reagent lots manufactured over a 2- to 4-year period and include 26 - 140 lots, depending on the analyte. For all assays, QC and panels were tested in multiple replicates on multiple runs. Clinical laboratory testing was performed in the Core Lab Facility at UCD Clinical Research Centre St. Vincent's University Hospital, Dublin Ireland. Here, QC was tested across multiple (minimum 3) reagent lots over 3 years for LH, FSH, Progesterone, Estradiol and Testosterone. All assays were run on the Abbott Architect platform. Multiple calibrator lots and, in some cases, QC and panel lots were used over the duration of analysis at both sites. 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 level for all assays was less than 10%CV when tested during manufacturing and in the clinical laboratory. Likewise, human serum panels tested during manufacturing had imprecision less than 10% for all assays on all panel levels.

Conclusions: Each of the fertility/pregnancy assays evaluated showed consistent reagent lot-to-lot performance on all control levels and human serum panels tested during manufacture and at St. Vincent's clinical laboratory. Reliable laboratory results give physicians the necessary information to put patients on the path to a healthy pregnancy.

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Analytical Performance Assessment of an Automated Research Use Only Immunoassay for Abbott's Alinity i and ARCHITECT i systems

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Introduction: Adrenocorticotrophic hormone (ACTH) is a polypeptide hormone consisting of 39 amino acids. ACTH is produced in the pituitary gland and regulates adrenal gland activity and cortisol levels in the body. This peptide hormone is derived proteolytically from proopiomelanocortin (POMC) precursor protein. The Research Use Only (RUO) Abbott Alinity i and ARCHITECT i1000SR ACTH dual mAb immunoassay has been developed for the quantitative detection of ACTH in human EDTA plasma.

Methods: The Alinity i and ARCHITECT i1000SR ACTH assay is a two-step sandwich immunoassay consisting of mouse and rabbit monoclonal antibodies. ACTH measurements are used in the differential diagnosis and treatment of certain disorders of the adrenal glands such as Cushing's syndrome, adrenocortical insufficiency, and ectopic ACTH syndrome. The analytical performance of the assay was assessed for analytical sensitivity, linearity, autodilution, precision, endogenous and drug interfering substances, specimen handling and preanalytics, and EDTA interference.

Results: Limits of Detection and Quantitation were < 0.5 pg/mL and < 1.0 pg/mL, respectively. Specimen dilution analysis yielded linear results across the Analytical Measuring Interval (AMI) of 3.7 - 1,500 pg/mL. A 1:10 autodilution protocol was generated for specimens exceeding the AMI resulting in an Extended Measuring Interval (EMI) of 1,500 - 15,000 pg/mL. Dilution recovery for samples within the EMI was within +\- 8% (%CV < 5%). Total within laboratory imprecision (20-Day) results were < 5.3%CV. Except for hemolysate, observed interferences of endogenous matrix components and drug interferences were <10%. Cross-reactivity for physiological ACTH precursor, POMC, and break-down products, alpha MSA and CLIP, ranged from approximately -0.03 to 1.07% similar to a comparator platform. The Abbott

ACTH assay appears robust to increased EDTA concentration that could result from short sampling during collections, whereas a comparator assay demonstrated ACTH concentration depression with increased EDTA. Similar to other comparator assays, ACTH in plasma was stable at 22°C +/- 3°C or refrigerated for up to 3 hours; ≤ -20°C for 30 days; and up to 1 freeze/thaw cycle.

Conclusions: An analytically robust RUO automated dual mAb immunoassay for Abbott's Alinity i and ARCHITECT i instrument systems.

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Analytical and Clinical Performance of the ADVIA Centaur Anti-thyroglobulin (aTgII) Assay*

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Background: Thyroglobulin (Tg) is a large, heterogeneous glycoprotein (molecular weight of 660,000 Daltons) found in the follicular cells of the thyroid. Thyroglobulin plays an important role in the biosynthesis of thyroid hormones T3 and T4. The measurement of autoantibodies against thyroglobulin (anti-Tg) is useful in differentiating patients with autoimmune thyroid disease. In patients with thyroid carcinoma, measurement of Tg antigen must consider the likelihood of the presence of significant levels of anti-Tg, since measurement and detection of Tg antigen may be influenced by the presence of anti-Tg antibodies. The objective of this study was to evaluate the analytical and clinical performance of the newly developed ADVIA Centaur® Anti-thyroglobulin II (aTgII) assay*.

Methods: The ADVIA Centaur aTgII assay is a fully automated, 1-step, analyte-bridging immunoassay using acridinium ester chemiluminescent technology for the detection of thyroglobulin autoantibodies. Analytical performance was studied in terms of precision, detection capability (LoB, LoD, and LoQ), interference, linearity, and hook effect. Clinical performance was established using a reference interval study and clinical concordance against a commercially available assay.

Results: Repeatability and within-laboratory imprecision ranged from 1.8 to 6.0% and from 4.2 to 7.7%, respectively. The assay has a limit of detection (LoD) of 1.3 IU/mL and a limit of quantitation (LoQ) of 1.4 IU/mL, with a linear measuring range up to 1000 IU/mL. Interference testing results showed all biases <6%, including biotin at 3500 ng/mL. There was no hook effect up to 50,000 IU/mL. A reference interval for the presence of anti-thyroglobulin antibodies was established using 198 apparently healthy males <30 years of age who had no personal or family history of thyroid disease and no history of autoimmune disease. The 95% nonparametric upper reference limit was calculated to be <1.3 IU/mL. A cutoff value for autoimmune thyroid disease was determined using a receiver operating characteristic (ROC) curve to optimize clinical agreement with the Beckman ACCESS Thyroglobulin Antibody II assay. A total of 237 patients (64 Hashimoto's disease, 41 Graves' disease, anemia patients, other thyroid disease patients, and non-thyroid autoimmune disease patients) and 56 healthy individuals were included in the study. With a cutoff value of 4.5 IU/mL, the positive percent agreement was 98.6% (71/72), with a 95% confidence interval (CI) of 92.5-100.0%. The negative percent agreement was 94.6% (209/221), with a 95% CI of 90.7-97.2%. The overall percent agreement was 95.6% (280/293), with a 95% CI of 92.5-97.6%.

Conclusion: The autoimmune cutoff of 4.5 IU/mL shows high positive, negative, and overall agreement with the Beckman ACCESS Thyroglobulin Antibody II assay. The analytical performance of the ADVIA Centaur Anti-thyroglobulin II (aTgII) assay shows acceptable sensitivity for the detection of thyroglobulin antibodies. *Under FDA review. Product availability varies by country and is subject to regulatory requirements.

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Performance Evaluation of the Atellica IM Thyroglobulin Assay

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Background: Quantitative measurements of thyroglobulin (Tg) are used to assess the effectiveness of treatment for thyroid cancer and monitor for recurrence. Patients who have been successfully treated for thyroid cancer by undergoing thyroidectomy are expected to have very low or undetectable Tg levels. Second-generation thyroglobulin assays with a functional sensitivity <0.05 ng/mL provide the opportunity to detect changes in Tg levels earlier and may allow patients to remain on thyroid replacement therapy (without TSH stimulation) prior to Tg testing. The purpose of this study was to evaluate the analytical performance of the Atellica® IM Thyroglobulin (Tg) Assay* (Siemens Healthcare Diagnostics Inc.) using human serum and plasma samples on the Atellica® IM Analyzer. **Methods:** The Atellica IM Thyroglobulin (Tg) Assay is a fully automated two-site sandwich immunoassay using direct chemiluminescent technology. Thyroglobulin reagents include an anti-Tg mouse monoclonal antibody labeled with acridinium ester in the Lite Reagent and a capture Solid Phase consisting of anti-Tg mouse monoclonal antibody bound to magnetic particles. Patient samples (100 µL for a single determination) are incubated with the Lite Reagent to form a Tg/acridinium ester complex. Solid Phase reagent and buffer are added and allowed to incubate, resulting in the formation of an acridinium ester/Tg/Solid Phase complex. Separation occurs, and the signal is proportional to the concentration of thyroglobulin in the sample. **Results:** The Atellica IM Tg Assay has a limit of quantitation ($LoQ_{20\%CV}$) of 0.041 ng/mL, with a linear measuring range up to 150 ng/mL. With automated dilution (1:50), the measuring range is extended to 7500 ng/mL. Equivalent results were obtained among serum, lithium heparin plasma, and EDTA plasma specimens. Reproducibility was assessed using the CLSI EP15-A3 protocol, with pools of serum specimens ranging from approximately 0.200 to 135 ng/mL. Repeatability %CVs ranged from 1.5 to 6.2%. Within-lab precision %CVs ranged from 3.0 to 9.8%. No interference was observed with 3500 ng/mL biotin. Split sample correlation between the Atellica IM Tg Assay and the Beckman ACCESS Thyroglobulin assay with serum specimens (n = 126) produced the following statistics by Passing Bablok analysis: slope = 1.26 and intercept = -0.145 ng/mL over a concentration range of 0.080 to >115 ng/mL. The Pearson correlation coefficient was 0.983. **Conclusion:** The Atellica IM Thyroglobulin Assay provides accurate and precise measurements of thyroglobulin and demonstrates second-generation functional sensitivity, with a limit of quantitation of 0.041 ng/mL. *Under development. Not available for sale. Features listed are development design goals. Future availability cannot be guaranteed.

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Performance Evaluation of the Glycated Hemoglobin Assay in Two Different Methods and Comparison with High Performance Liquid Chromatography HPLC

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Background: Diabetes Mellitus continues to grow worldwide, motivated by a complex interaction of socioeconomic, demographic, environmental and genetic factors. According to the International Diabetes Federation, this increase is largely due to type 2 diabetes and related risk factors. However, the numbers of type 1 diabetes in childhood are also growing. In addition to the rapid diagnosis of the disease, a seriousness of complications makes continuous monitoring of the diabetic patient extremely necessary. The glycated hemoglobin assay (HbA1c) is widely used for both situations, as it represents the patient's glycemic status in the last 2 to 3 months. The aim of this study is to evaluate the performance of the new Atellica CH Analyzer enzymatic HbA1c assay, and to compare it with the previous methodology (turbidimetry) and the reference method (HPLC).

Methods: In the study, the Atellica CH™ - Siemens Healthineers Analyzers with the enzymatic (A1c_E) and turbidimetric (A1c_3) reagents were used, and Variant II Bio Rad® as reference methodology. The precision study was performed according to EP15-A3 with two levels of commercial quality control and four medical decision pools (MDP), method comparison according to EP09 using at least 160 samples and hemoglobin variants interference evaluation with the four main variant types (HbC, HbD, HbE and HbS).

Results: The results of A1c_E on Atellica CH Analyzer are in accordance with the NGSP quality specifications (CV <3.5%) with a $\%CV_R$ from 0.369% to 0.943%, and $\%CV_{WL}$ from 0.397% to 1.628%. The A1c_E method comparison study with Variant II BioRad and A1c_3 showed an R^2 0,9920 with linear regression of Y = 0,998 * x + (-0,127), and an R^2 0,9858 with linear regression of Y = 0,992 * x + (-0,393), respectively. The mean was from 6.79 (Variant II) and 6.64 (A1c_E), suggesting that both methods are similar in a significance level of 5%. The bias estimative obtained between A1c_E and Variant II was 2.23% and mean TE of 4.35%, being inferior to the NGSP recommendations (5,0%).

Conclusion: The A1c_E assay showed consistent analytical performance according to the new NGSP guidelines (2019), which is essential to ensure confidence in the diagnosis and monitoring of diabetic patients, in addition to the methodology considered to be the reference. The comparison study demonstrated that the A1c_E assay has good agreement with both methods.

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Evaluation of Low TSH Levels according to the hCG Concentration in Pregnant Women

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Background: Pregnancy has a deep impact on the thyroid gland and its function, so that interpretation of laboratory testing differs from the non-pregnant patient. In the first trimester, when human chorionic gonadotropin (hCG) concentrations are highest, there is a transient suppression of serum thyrotropin (TSH). In normal pregnancy, TSH levels generally remain within non-pregnant reference intervals. However, in some patients TSH is suppressed and it has been suggested that hCG levels are related to this suppression. The objective of this study was to evaluate the relationship between low TSH levels according to the hCG concentration in pregnant women.

Methods: We analyzed blood samples requesting serum hCG and TSH levels from patients admitted to a private reference clinical laboratory in Brazil, from January 2016 to December 2019. Anonymized data on laboratory tests was available from a database of the local Laboratory Information System. We separated patients according hCG concentration (IU/L, Electrochemiluminescence, Modular, Roche): G1 - 25,000 to 50,000; G2 - 50,000 to 75,000; G3 - 75,000 to 100,000; G4 - 100,000 to 200,000; G5 - 200,000 to 300,000; and G6 - higher than 300,000. All patients had TSH levels lower than 4,3 mIU/L (Electrochemiluminescence, Modular, Roche).

Results: We analyzed the results of 3610 pregnant women: G1 - 1076, G2 - 761, G3 - 622, G4 - 1033, G5 - 103, G6 - 15. The TSH median in the six different hCG groups were, respectively: 1.70; 1.49; 1,41; 1,18; 0,80 and 0,51 mIU/L; the difference among the groups was significant ($p < 0.05$), with lower TSH medians in higher hCG levels. The percentage of TSH levels lower than 0.4 mIU/L in the hCG groups were, respectively: 1.6; 2.2; 3.9; 11.0; 28.2 and 40.0%, with a significant difference among the groups with hCG higher than 50,000 IU/L ($p<0.05$). The percentage of TSH levels lower than 0.1 mIU/L in the hCG groups were, respectively: 0.7; 0.4; 0.6; 1.7; 11.0 and 20.0%, with a significant difference among the groups with hCG higher than 75.000 IU/L ($p<0.05$), except with the G6 (hCG > 300,000), possibly due to the sample size of this group.

Conclusion: We found a inverse correlation between TSH and hCG, with higher percentage of low TSH levels in patients with higher hCG concentration. This information helps clinicians to interpret TSH levels in pregnant women.

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Hemoglobin A1c Monitoring by Bio-Rad, Beckman, and Roche Analyzers.

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Background: Ion exchange high performance liquid chromatography (HPLC) has been used for monitoring hemoglobin A1c (HA1c) with throughput typically longer than immunoassays. Recent generations of immunoassays require less time and expertise. However, immunoassays may be more susceptible to interference such as high lipids levels, resulting in HA1c variations by using turbidimetric measurement. The purpose of this study was to compare the results obtained with ion exchange HPLC and two immunoassay instruments (Beckman and Roche), and to assess variations due to hyperlipidemia in our selected patient samples.

Methods: We investigated the correlation between Bio-Rad Variant II Turbo at Wake Forest Baptist Hospital, with the Roche c513 at University of Rochester Medical Center, and the Beckman-Coulter DXC 700 AU at High Point Medical Center. Both sets of data consisted of 120 samples, 40 % of which had elevated triglyceride levels in excess of 400 mg/dL in the comparison with the Beckman instrument. Samples for the Bio-Rad were automatically diluted and injected into the analytical cartridge, and the HA1c fraction was automatically calculated based on absorbances of all hemoglobin peaks. Samples for the Beckman-Coulter were automatically pretreated, without additional manual pretreatment or washing. The red cells were then lysed, and the total hemoglobin and HA1c were measured, colorimetrically, and immunoturbidimetrically, respectively. The samples analyzed on the Roche instrument were run automatically without premixing, in a similar manner to the Beckman instrument.

Results: Comparison was made using a two instrument comparison with the greatest allowable error set at +/- 6% consistent with current College of American Pathology (CAP) guidelines. 95% of samples were required to be within the allowable error in order to pass. HA1c levels on the Bio-Rad Variant II Turbo ranged from 4.8% to 16.8%. The Beckman-Coulter DXC 700 AU showed good correlation with the Bio-Rad ($R = 0.995$). The average error index was 0.16, with an error index range of -0.94 to 1.36. 117 of the 120 samples were within the allowable error. Likewise, the Roche c513 showed a strong correlation with the Bio-Rad ($R = 0.995$). The average error index was 0.03, with an error index range of -0.79 to 0.91. All of the 120 samples were within the allowable error of +/- 6%. HA1c levels on the Bio-Rad Variant II Turbo ranged from 4.6% to 14.3% in the data set compared with the Roche instrument.

Conclusion: Monitoring of HA1c Beckman-Coulter DXC 700 AU was well correlated to the Bio-Rad Ion exchange HPLC. For our two sample sets, the high correlation with hyperlipidemic samples suggests that the Beckman-Coulter DXC 700 AU does not show significant interference from hyperlipidemia, and that this instrument is appropriate to use in these cases. The Roche c513 also passed, confirming that it is a viable instrument for measuring HA1c, as well.

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Repeated Hypoglycemic Episodes in a Patient with Type 1 Diabetes: Considerations in Insulin Measurement

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Case report: A 16-year old male with 7-year history of Type I diabetes had multiple admissions to the PICU for hypoglycemic episodes in the past 2 months. His home medications included insulin glargine and Humulin R. During the most recent hospital stay, his c-peptide concentration was non-detectable and insulin results were shown in Table 1A. His hypoglycemic symptoms and low blood glucose (<60 mg/dL) persisted despite sugar intake and glucagon treatment. Background: Exogenous insulin administration is a common cause of hypoglycemia in patients with Diabetes Mellitus. Laboratory testing of insulin and c-peptide can be a crucial part of the investigation during a hypoglycemic attack. However, standard insulin immunoassays have shown various cross-reactivities with insulin analogs. Therefore, we analyzed three insulin analogs and two types of unmodified human insulin by three commercial immunoassays, including the in-house Abbott Architect assay. Methods and results: One vial of each of the three insulin analogs (glargine [Lantus®], detemir [Levemir®], Insulin lispro [Humalog®]) and two regular human insulin (Humulin R and Humulin N) were obtained from our hospital pharmacy. Each was diluted with a serum pool to final insulin concentrations of 75 and 400 mIU/L. The percentage cross-reactivity was calculated from the ratio of the measured and calculated concentration. A summary of the recoveries is presented in Table 1B. Conclusion: Different commercial assays demonstrated large variability in insulin analog cross-reactivities. Given the lack of detailed information in the package insert, individual clinical laboratory should perform its own study. In addition, a combination of commercial assays could be useful to estimate concentrations of insulin analogs. In our case, based on clinical presentation and laboratory results, it was suspected that the hypoglycemia was secondary to incorrect insulin dosing. After dose adjustment, the patient improved clinically and was discharged 3 days later.

Table 1A: Insulin results measured with Abbott Architect and Roche Cobas.		
	Abbott Architect (µIU/mL)	**Roche Cobas (µIU/mL)**
03:26 At admission	448	21.2
12:00	111	9.5
06:03 On second day	69	5.7
Reference range	<26	2.6-24.9

Table 1B: Cross-reactivites of insulin analogs and unmodified human insulin.			
	Cross reactivity, %		
Analog/human insulin and concentration	**Abbott Architect**	**Siemens Centaur**	**Roche Cobas**
Insulin glargine (Lantus@)-sequence: GIVEQCCTSICSLYQLENYC**G** FVNQHLCGSHLVEALYLVCGERGFFYTPKT**RR**			
75 mIU/L	119	133	14
400 mIU/L	105	89	8
mean	112	111	11
Insulin detemir (Levemir@)-sequence: GIVEQCCTSICSLYQLENYCN FVNQHLCGSHLVEALYLVCGERGFFYTPK-**C14**			
75 mIU/L	134	93	3
400 mIU/L	132	60	<1
mean	133	77	NA
Insulin lispro (Humalog @)-sequence: GIVEQCCTSICSLYQLENYCN FVNQHLCGSHLVEALYLVCGERGFFYT**KPT**			
75 mIU/L	140	185	4
400 mIU/L	113	123	<1
mean	127	154	NA
Regular human insulin (Humulin R)-sequence: GIVEQCCTSICSLYQLENYCN FVNQHLCGSHLVEALYLVCGERGFFYTPKT			
75 mIU/L	94	116	130
400 mIU/L	86	84	95
mean	90	100	113
NPH human insulin (Humulin N)-sequence: GIVEQCCTSICSLYQLENYCN FVNQHLCGSHLVEALYLVCGERGFFYTPKT			
75 mIU/L	105	100	111
400 mIU/L	93	76	83
mean	99	88	97

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Development of an LC-MS/MS Assay for the Analysis of Endogenous Cannabinoid Plasma Concentrations

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Background: The discovery of cannabinoid (CB) receptors and the growing popularity of cannabis use has sparked interest in understanding the biological function of CB receptors' endogenous ligands, or endocannabinoids (eCBs). Most attention has focused on the physiological role of two eCBs, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG). THC and CBD are capable of functioning as CB1 and CB2 agonists and, therefore, impact important physiological processes. Widespread recreational and medical use of cannabis and CBD products underscores the importance of understanding how exogenous cannabinoids effect the endocannabinoid system. To address this, liquid chromatography - multiple reaction monitoring mass spectrometry (LC-MRM) assays have been developed to quantify AEA and 2-AG in patient plasma. **Methods**: Detection of the eCBs was optimized by direct infusion ESI-MS/MS (Waters, TQ-S micro) of pure standard compounds (Cayman). Both compounds performed best in positive ion mode and at least two precursor and product ion pairs (quantifier and qualifier ions) were selected to monitor each compound. Cone voltage and collision energy optimization was performed for ion transitions monitored. Separation of analytes was achieved using reversed-phase liquid chromatography on a Waters Acquity UPLC equipped with a BEH C18 column (2.1 ID x 50 mm, 1.7 um particles) and mobile phases A and B consisting of 5 mM ammonium formate in water with 0.1% formic acid and acetonitrile with 0.1% formic, respectively. A 3.5 min gradient was used with a total run time of 5 min per sample. **Results:** Collection tube type was evaluated and did not grossly affect the measured concentrations of AEA. However, the measured concentrations of 2-AG varied up to 5-fold depending on collection tube type. Based on stability of 2-AG, K-EDTA was selected as the collection tube of choice. Assay linearity was determined for each analyte using a seven point calibration curve. The linear ranges for AEA and 2-AG quantification in plasma are 0.05 to 25 ng/mL and 0.25 to 25 ng/mL, respectively. High, mid, and low concentration samples (4.0 ng/mL, 1.0 and 0.6 ng/mL) were analyzed to assess accuracy and precision. The observable bias for the high, mid, and low spiked samples were measured. In addition, stability in the auto-sampler was assessed at 24, 48, and 96 hours. Results demonstrate that AEA and 2-AG are stable in the LC auto-sampler for up to 24 hours. Sample carryover was evaluated and none was observed following injection of the highest calibrator (25 ng/mL). Within run accuracy and precision was determined in stripped plasma, indicating that calibrator and QC bias was < 20% and coefficient of variations (%CV) were <10%. Matrix effect studies revealed no matrix effects for AEA but significant interpatient matrix effects for 2-AG. **Conclusion:** These LC-MRM assays will be applied to participant plasma in clinical trials assessing the therapeutic efficacy and safety of CBD therapy. Measurements of AEA in patient plasma have been validated for accuracy, precision and a lack of significant matrix effect. However, the measurement of 2-AG still presents with significant pre-analytical challenges and matrix effects that should be carefully considered before measuring 2-AG levels in clinical trials.

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Effect of Renal Function on LC-MS/MS Measurement of PTHrP and Its Correlation to Calcium and PTH Concentrations

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Background: Parathyroid hormone (PTH) and parathyroid hormone related peptide (PTHrP) both act to regulate calcium concentrations. High concentrations of PTHrP may indicate hypercalcemia of malignancy and can prompt investigation into a new or recurring cancer. Early studies using PTHrP radioimmunoassays (RIA) revealed elevated PTHrP concentrations in normocalcemic renal failure patients. The specificity of these early assays for the C-terminus of PTHrP may explain elevated concentrations in these patients. At our institution, we developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for PTHrP targeting a peptide in the middle of the PTHrP sequence (to avoid measuring terminal fragments). Given the high specificity of LC-MS/MS, our objective was to revisit the earlier observation that PTHrP concentrations may be elevated in renal failure patients. Methods: We used retrospective chart review to investigate 1) whether PTHrP concentrations differed between adult patients with and without renal impairment and 2) the association between PTHrP, PTH, and calcium concentrations in these patients. After exclusion of patients with cancer, the participants (n=107, 20-90y, 56% female) were categorized based on eGFR using the 2009 CKD-EPI equation following KDIGO guidelines. We focused on patients with healthy kidney function (n=21, 20-73y, 43% female), stage 4 kidney disease (n=40, 23-90y, 63% female), and end stage renal disease (ESRD, n=19, 27-81y, 58% female), 6 of whom were on hemodialysis. Results: When measured by LC-MS/MS, we observed higher PTHrP concentrations in ESRD and stage 4 patients compared to those with healthy kidney function ($p<0.0001$ for both). Overall, there was a strong negative correlation between eGFR and PTHrP ($\rho=-0.743$, $p<0.0001$). In contrast to the previous study documenting elevated C-terminal fragments of PTHrP in normocalcemic patients, we observed that 78% of patients with PTHrP concentrations above the sex-specific reference interval had hypercalcemia, in agreement with the positive association between concentrations of PTHrP and calcium ($\rho=0.352$, $p=0.0003$). Of the 11 patients with elevated PTHrP and normal calcium, 6 had presented with hypercalcemia and had been treated prior to the PTHrP sample being drawn. No statistically significant difference was observed between distributions of PTHrP concentrations in stage 4 and ESRD patients with and without hypercalcemia. Among ESRD patients, higher PTHrP concentrations were observed in patients on dialysis compared to those not on dialysis ($p=0.003$). Therefore, our data suggest that elevated PTHrP concentrations in these populations are not solely due to decreased glomerular filtration. For those hypercalcemic patients that had both PTH and PTHrP measured (n=46), 50% of patients with elevated PTHrP had appropriately suppressed PTH and 7% had either primary or secondary hyperparathyroidism. Conclusion: Considering the specificity of the LC-MS/MS method for the central portion of PTHrP, we conclude that elevated PTHrP concentrations may occur in patients with severe renal dysfunction. PTHrP elevations correlate with hypercalcemia in the majority of these patients, suggesting that the measured PTHrP is functional, and not a biologically inactive terminal fragment. Clinicians should be cognizant of the method used to measure PTHrP when evaluating hypercalcemia, particularly in patients with renal insufficiency.

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Rapid UPLC-MS/MS Dried Blood Spot Analysis of Steroid Hormones for Clinical Research

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Background: Dried Blood Spots (DBS) are an established microsampling technique providing a low-cost approach of collecting, shipping and analyzing samples for clinical research. Ligand-binding assays (LBAs) are often used as the frontline testing methodologies for DBS samples in steroid hormone analysis. Although rapid, the relatively low analytical specificity of the LBAs may necessitate follow-up, using liquid chromatography - tandem mass spectrometry (LC-MS/MS). The challenge is to create a fast, analytically sensitive and selective LC-MS/MS methodology.

Methods: Certified androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol reference material purchased from Sigma Aldrich (Poole, UK) were used to create calibrators and QC materials in whole blood; prepared by mixing 50/50 (v/v) red blood cells from BioIVT (West Sussex, UK) and MSG4000 stripped serum from Golden West Biologicals (CA, USA). Blood spots were prepared by adding 50µL whole blood calibrators and QCs to Whatman 903 Protein Saver Blood Spot cards from Sigma Aldrich (Poole, UK) and then left to dry. Two 3mm DBS samples were pre-treated with an internal solution and mixed for 5 minutes. Solid Phase Extraction (SPE) was carried out with a Waters Oasis™ MAX µElution 96-well plate, 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 2.1mm x 50mm CORTECS™ C_{18} 2.7µm column with pre-column CORTECS C_{18} 2.7µm VanGuard™ using a water/methanol/ammonium fluoride gradient and quantified with a Xevo™ TQ-S micro mass spectrometer.

Results: The method enabled rapid separation in 1.4 minutes (2.3 minutes injection to injection) for 17-OHP, androstenedione, cortisol, 11-deoxycortisol and 21-deoxycortisol with baseline resolution of steroid isobars. Calibration lines were linear from 0.5 - 500 ng/mL for androstenedione and 11-deoxycortisol; and 1.0 - 500 ng/mL for cortisol, 17-OHP and 21-deoxycortisol with correlation coefficients (r^2) >0.99 over five occasions. Coefficients of variation (CV) for total precision and repeatability over five occasions at four concentrations; 2, 5, 50 and 400ng/mL, were ≤ 9.3% (n = 25) with accuracies ranging from 94 - 110%.

Conclusion: The challenge was met by using Ultra Performance Liquid Chromatography (UPLC™) combined with CORTECS™ 2.7µm particle columns to provide UPLC separations at high linear velocities with minimal loss in column performance. This offline automated method demonstrates excellent linearity, analytical sensitivity, precision and accuracy, while providing high sample throughput capabilities for the analysis of androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol in dried blood spots for clinical research purposes.

For Research Use Only, Not for use in diagnostic procedures.

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An Unusual Cause of High Serum Prolactin

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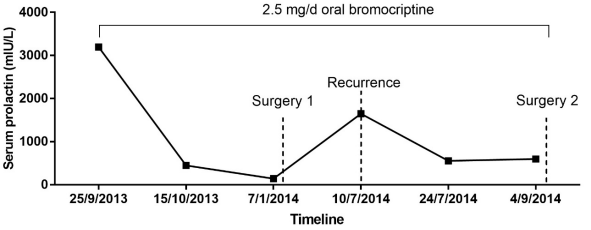
Background Nonpuerperal mastitis refers to all causes of inflammatory changes in female breast and mammilla which are not related to lactation. Plasma cell mastitis (PCM) is one type of nonpuerperal mastitis and it often occurs during non-childbearing age. X-ray, ultrasound and pathologic examination are commonly used for PCM diagnosis. Here, we report a case of a PCM female patient with repeated high levels of serum prolactin (PRL), and discuss the potential value of PRL in PCM occurrence. **Methods and Results** A 45-year-old female, with no significant past medical history, presented with high serum prolactin (151 ng/mL, reference interval 3-27 ng/mL). Oral bromocriptine (2.5 mg/d) was prescribed. Later, she noted a breast lump, which progressively increased in size, became painful, was accompanied by redness and swelling, and had a milky white nipple discharge. The lump was excised; pathology revealed plasma cell mastitis. Six months later, she noted a second lump, which was excised and also revealed plasma cell mastitis. **Conclusion** PCM accounts for 4%-5% of breast benign lesions in which the most annoying problem is recurrence with unknown causes. At the moment, a prospective study is on-going to investigate the timing and condition of serum PRL to initiate nonpuerperal mastitis, or more precisely PCM. If it turns out to be true that PRL triggers the onset of PCM, it will emphasize the urgent need for indispensable detection of serum PRL in non-lactational women health care.



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Analytical Performance Assessment of Atellica Solution Thyroid Assays using Sigma Metrics

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Background: Thyroid dysfunctions can happen at any stage of life, impairing the regulation of the function of important organs such as the heart, brain, liver and kidneys. With the increase in life expectancy and the number of elderly people, whose prevalence of thyroid dysfunction diseases appears between 2 to 4% of individuals over 65 years and in 0.5 to 1% of the general population. Several factors are involved in the genesis of thyroid dysfunctions, among which are the autoimmune mechanisms, genetic factors related to the HLA system and environmental factors. Reinforcing that, for an adequate diagnosis and monitoring of these diseases, laboratory tests are essential. In this scenario, the authors of this study aimed to validate the performance of the main assays of the thyroid profile in a large laboratory operating in Brazil. We apply Sigma's metric measures to assess the Atellica Immunoassay platform, in order to propose a methodological implementation plan in this company and compare to similar studies. **Methods**: For the assays Thyroid Stimulating Hormone 3-Ultra (TSH), Total Thyroxine (T4), Total Triiodothyronine (T3), Free Triiodothyronine (FT3) and Free Thyroxine (FT4) on the Atellica IM 1600 Siemens Healthineers Analyzer, the evaluation of the verification of the imprecision and estimation of bias by peer group were performed by the within-run imprecision($\%CV_R$) and within-laboratory imprecision ($\%CV_{WL}$) with a total of 25 replicates per QC sample for each assay. Method comparison studies were performed between the Atellica IM and Architect Abbott assays according to EP09, using a minimum of 40 serum samples that covered the entire assay linearity range. For the evaluation of sigma metrics, TEa goals from RiliBÄK was used as a reference. **Results**: The imprecision study agree with the specifications of the analytical quality. The $\%CV_R$ was 0.962% to 3.006%, $\%CV_{WL}$ was 1.70% to 3,90% and the bias was 0.41% to 10.43%. All QC levels for all tests were higher than 3 Sigma, with 7 out of 10 levels were 6 Sigma or higher (world class) and 3 out of 10 levels were 4 and 5 Sigma (good). **Conclusion**: All assays tested on the Atellica IM Analyzer demonstrated consistent results of imprecision, bias and Sigma, according to the quality specifications. Despite conducting the study of sigma metrics in this work, it is worth mentioning that there is still no consensus on the appropriate source of ETa which makes any result of the Sigma metric calculations complex to interpret. Our conclusion demonstrates that it is necessary know the performance of each assay and apply more realistic ETa before implement the periodic evaluation of Sigma metrics.**Siemens Healthineers supported the studies by providing systems, and reagents.*

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Evolution of Changed Testosterone Results in Female Patients in a Laboratory of Clinical Analysis

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Background Hormone replacement with testosterone in women is not recommended by societies of medical specialties, in view of the increased risk compared to the benefits about libido, well-being or aesthetics. However, this practice has been widely used. The authors evaluated the drugs used by female patients who measured total testosterone, in order to verify the percentage of women who had an increase in testosterone due to hormone replacement; analyze what were the compounds and routes of administration by which these substances were administered; and observe the levels of total serum testosterone while using these medications.

Patients and methods Were studied 9929 female patients who measured total testosterone for one month in a private laboratory from Rio de Janeiro, Brazil, aged 20 to 80 years old. Testosterone was measured by a competitive chemiluminescence assay (Roche diagnostic Ltd). In cases of elevated testosterone levels, the standard procedure of the laboratory is to contact the patient and/or the assistant doctor before the release of the result, to obtain information about the use of medications, doses and route of administration.

Results Of the total of studied patients, 388 (3.9%) had high total testosterone. Of these, it was possible to contact 207 doctors or patients to obtain information on probable causes of the increase in total testosterone: 153 (73.9%) were post-menopausal, with a mean age of 66.1 years and 54 (26.1%) were of childbearing age. Of the 207 patients contacted, we observed that: 105 (50.7%) used products containing testosterone or similar; 50 (24.2%) were investigating for hyperandrogenism; 6 (2.9%) used contraceptives containing progestogens derived from 19-nor-testosterone; in 46 (22.2%) there was no apparent cause for elevated testosterone from the information obtained. The most used medications and doses administered were Propionate gel: 2-5%/g/day; Intramuscular cypionate: 25-100 mg/week; Oral undecanoate: 40-80 mg/day; Subcutaneous testosterone implants (without information about dosage); Testosterone-containing vaginal cream: 0.25 mg/day; and several products called "bioidentical testosterone". All products led to testosterone levels above the reference values for the method, from 8.4 to 48.1 ng/dL for women aged 20 to 49 years and from 2.9 to 40.8 ng/dL for those with more than 50 years, with levels between 62.5 and 900 ng/dL. **Conclusion** Prescribing high doses of testosterone has been a growing practice among physicians and that, even when not prescribed, patients have been using it without medical advice. So-called natural substances appear to be contaminated with testosterone. Almost a third of the patients were of childbearing age. This group is subject to the same risks as older women, and may also have an impact on fertility or even on the fetus if they become pregnant.

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Putative Roles for Fetuin A in Micro- and Macro-Vascular Complications of Type 2 Diabetes Mellitus

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Background: Fetuin-A, an anti-inflammatory glycoprotein synthesized in the liver, inhibits insulin action, apoptosis of vascular smooth muscle cells and prevents heterotropic calcification. Low Fetuin-A concentration has been shown to be associated with increased arterial calcification and overproduction of inflammatory cytokines. This study evaluates the relationship between serum Fetuin-A, insulin resistance (IR), and micro- and macro-vascular complications in patients with type 2 diabetes mellitus (T2DM). **Methods**: Fasting serum Fetuin-A, lipid profile and high-sensitivity C-reactive protein (hs-CRP) were measured in 201 (69M, 132F) T2DM patients. Anthropometric and clinical data were recorded and subjects were classified on the basis of the degree of adiposity and homeostasis model assessment of insulin resistance (IR). Ratio of urine microalbumin to creatinine was determined to categorize subjects as normo- (NAO, ratio < 30mg/g); micro- (MIA, ratio 30-300mg/g) and macro-albuminuric (MAA, ratio > 300 mg/g). Univariate and multivariate analyses were used to compare study subjects and binary logistic regression (Odds Ratio (OR)) analyses were used to evaluate associations with micro- and macro-vascular complications. **Results**: Fetuin A was significantly higher in subjects with IR > 2 compared to subjects with IR < 2 (350 vs 331 ug/ml). Fetuin A was significantly ($p < 0.05$) correlated with Total Cholesterol (r = 0.22); LDL-cholesterol (r= 0.22); Triglycerides (r= 0.29); ALT (r=0.28) and inversely correlated with age (r= -0.19). Fetuin A was significantly lower in subjects with ischaemic heart disease (IHD) (310 vs 331 ug/ml); (MIA and MAA (308 vs 340 ug/ml), sensory neuropathy (SN) (318 vs 348 ug/ml); autonomic neuropathy (AN) (305 vs 337ug/ml) and retinopathy (319 vs 350 ug/ml) compared to T2DM patients without these complications. Binary logistic regression showed significant associations with MIA and MAA (OR = 0.993); AN (OR = 0.995); SN (OR = 0.991), retinopathy (OR = 0.991) and IHD (OR = 0.993). **Conclusions**: The cardiovascular implications of our findings are that lower Fetuin A could predispose to arterial calcification which is common in T2DM in association with neuropathy and nephropathy. Estimation of Fetuin A could be used to identify patients at risk of these adverse prognostic complications that are also independent risk factors for cardiovascular disease and all-cause mortality.

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Evaluating the Siemens Thyroid Stimulating Immunoglobulin (TSI) Assay in the Diagnosis and Prognosis of Graves' Disease: An Update

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Introduction: Graves' disease (GD) is an autoimmune condition that is the most common cause of hyperthyroidism. GD is caused by autoantibodies that bind and stimulate the thyroid stimulating hormone receptor (TSHR), such that the production of thyroid hormones is under the control of these autoantibodies rather than TSH. Currently, laboratory testing for GD includes measuring these TSHR antibodies (TRAbs). However, since there are both stimulating and blocking TRAbs, these assays are not specific for those that cause GD. This ongoing study aims to evaluate the Siemens TSI assay, which specifically measures levels of stimulating autoantibodies, compared to the currently available Roche TRAb assay.

Methods: Two hundred hyperthyroid patients will be enrolled in this single-center prospective, multiphase study. Study patients are under the care of an endocrinologist at a University of Calgary clinic, and are approached by the endocrinologist to participate if they have suspected GD. Consent is obtained, and along with standard of care including TRAb measurement, an additional blood sample is collected for TSI measurement. Samples for TSI measurement are frozen and batch tested in duplicate locally using the Siemens TSI assay on the Siemens Immulite (Siemens Medical Solutions). Samples for TRAb measurement are referred out for testing, and are tested on the Roche Cobas 8000 platform (Roche Diagnostics). The clinical diagnosis of GD is made by the endocrinologists who are blind to TSI levels.

Results: To date, a total of 96 hyperthyroid patients have been enrolled in the study, including 74 (77%) patients with GD. The TSI and TRAb assays were correlated (R^2=0.71, $p<0.0001$) and had 93% concordance. For the primary analysis, patients taking anti-thyroid medication (ATD) at time of sample collection were excluded (n=14). Using manufacturer's specified cut-offs, the respective sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) for TSI was 98, 86, 95 and 95%, which were comparable to those generated by TRAb (97, 95, 91, and 98%). In patients with orbitopathy or goiter (i.e. clinical findings of GD, n=38), the assays performed identically, with a sensitivity, specificity, NPV, and PPV of 97%, 80%, 80%, and 97% respectively. In patients without orbitopathy or goiter (n=44), the TSI assay had perfect sensitivity and excellent specificity of 100% and 88% respectively (TRAb had 96% sensitivity and 100% specificity). When all hyperthyroid patients were included in the analysis (n=96), sensitivity, specificity, NPV and PPV of both the TRAb and TSI assays decreased slightly (TRAb: 89, 95, 72, 99%; TSI: 85, 86, 63, 95%). Notably, almost all (10/11) patients with false negative TSI results were on ATDs at the time of sample collection.

Conclusion: These new results indicate that TSI is an excellent biomarker of GD, particularly in the context of active, untreated GD. The TSI assay performs comparably to that of TRAb. Importantly, the prognostic ability of this assay to predict GD remission will be assessed in the next phase of this study, for which active recruitment is ongoing.

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Associations between BMI and Glycated Albumin in an Overweight and Obese Caucasian Population

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A number of studies have shown a weak to medium negative correlation between BMI and glycated albumin (%GA), but not between hemoglobin A1c (A1c) and BMI. Explanations proposed include chronic inflammation and differences in insulin secretion, affecting albumin production. However, many of these studies are limited by a narrow range of BMI. Using baseline samples from a clinical trial conducted by Virta Health for patients diagnosed with type 2 diabetes (75%) or prediabetes (25%), we have examined these relationships and possible underlying factors. This population was 95% Caucasian with a median BMI of 38.2 kg/m^2 (24.8-86.9 kg/m^2) and median age of 55 years (27-66 years). %GA was measured using kits from Diazyme and Roche on a Roche c501. All other analytes were measured on the same platform with Roche kits. Subjects with eGFR ≤60 mL/min/1.73m^2 and hemoglobin <12.0 g/dL were excluded from analysis. Spearman's correlation (N=386) showed no significant correlation between BMI and %GA or A1c at an α=0.05. However, there were moderate, sta-

tistically significant correlations (Spearman's rho, p-value) between BMI and %GA/A1c (-.205, p<.0001); glycated serum protein, GSP (-.158, p=.003); albumin (-.332, p<.0001); C-reactive protein, CRP (.395, p<.0001); and insulin (.238, p<.0001). Since both GSP and albumin individually show negative correlations with BMI while the %GA does not, this could suggest differences in both clearance of GSP and production of albumin. We then grouped subjects into three BMI categories and performed Kruskal-Wallis analysis. Differences are seen between these BMI categories for all variables except %A1c (Table 1). In summary, we have not found a significant correlation between %GA and BMI in this population. However, there are statistically significant differences in GSP, %GA, insulin, and albumin across BMI categories. Lower levels of GSP observed in the higher BMI groups could result from increased clearance of glycated proteins compared to nonglycated.

Table 1. Comparisons Across BMI Cohorts

Analyte (units)	Median (Range)		
	Group 0 BMI <30 N=33	Group 1 BMI 30 – 40 N=179	Group 2 BMI >40 N=145
GSP (µmol/L)	321 (193 - 712)	259 (135 - 581)	254 (130 - 658)
*	*13.5 (p=.001)*		
Albumin (g/dL)	4.6 (3.8 – 5.4)	4.5 (3.9 – 5.2)	4.4 (3.6 - 5.2)
*	*31.9 (p<.0001)*		
Insulin (µIU/mL)	14.4 (3.4 – 96.1)	20.4 (5.9 – 122.6)	24.6 (2.5 -209.5)
*	*27.2 (p<.0001)*		
%GA	16.0 (11.4 - 32.8)	13.5 (9.1 - 27.7)	13.8 (8.8 - 31.7)
*	*10.0 (p=.007)*		
A1c (%)	7.1 (5.6 – 12.0)	6.6 (5.4 – 12.2)	6.9 (5.5-12.5)
*	*3.4 (p=.186)*		

#Starting with N=386, 29 were removed due to missing BMI.
*Kruskal-Wallis Test Statistic (p-value).

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An Investigation of Positive Bias in Our Mass Spectrometry-Based 1α,25-Dihydroxyvitamin D Assay

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Background: A 1α,25-dihydroxyvitamin D (DHVD) test is used to assess the level of the biologically active form of vitamin D in patients with chronic renal disease and for differential diagnosis of hypercalcemia. The DHVD assay is challenging because there are numerous isomers present in patient specimens, and the active form has very low circulating concentrations (picomolar). Our hospital is currently using a Cookson-type triazolinedione derivatization protocol followed by LC-MS/MS quantification to determine 1α,25-dihydroxyvitamin D_2 and 1α,25-dihydroxyvitamin D_3 levels in serum specimens (1). While investigating a positive comparison bias for DHVD assay results, we discovered that the reference laboratories had adopted a new methodology. We thus modified our methodology to address these discrepancies. **Methods:** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the "gold standard" for quantification of vitamin D metabolites in complex matrices. Our original method started with solid-phase extraction and derivatization using 4-phenyl-1,2,4-triazoline-3,5-dione. The derivatized complex mixtures were chromatographically separated on Acuity BEH C18 columns (1.7 µm, 2.1x100 mm) using a Shimadzu UHPLC system coupled to a Sciex Qtrap 5500 instrument operating in triple quadrupole mode. Recent studies showed that immunoaffinity purification may reduce an interference metabolite that was co-eluting with DHVD peaks. However, we optimized liquid chromatographic separation to resolve interference peaks that may lead to positive bias of the DHVD results. **Results:** Our recent in-house alternative proficiency tests showed a positive bias in comparison to DHVD send-out test. Specifically, the results of our calibrators were acceptable, while the majority of patient specimens showed positive bias. We performed a thorough investigation, ruled out assay issues, and finally determined that the change was likely not within our lab. Two months later, the reference laboratories reported a modified sample preparation method that incorporated immunoaffinity isolation steps. Their decreased DHVD levels in patient specimens were consistent with the protocol change. These results indicated that our method was likely overestimating DHVD if there was an interference that may be co-eluting with the targeted analyte. Recent studies showed that 4β,25-dihydroxyvitamin D and 1α,25-dihydroxy-3-epi-vitamin D have the same mass-to-charge ratio as 1α,25-dihydroxyvitamin D and may cause bias in the quantification of DHVD. Based on the retention time, we suspected that an interference peak was possible due to the presence of 4β,25-dihydroxyvitamin D (2). By using a phenylhexyl LC column and slightly extending the LC gradient, we were able to separate an interference peak from the DHVD peak. The assay results using our updated LC-MS/MS method were consistent with results from the reference laboratory. **Conclusion:** We identified the root cause of positive bias in our DHVD assay. Optimizing chromatographic separation allowed us to address the deviation in a timely manner. Our quantification assay will be revalidated after the method modification.

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A Higher Cutoff for TSI Would Better Predict Recurrence in Patients with Graves' Disease?

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Background Graves disease (GD) is an autoimmune disease mediated by immunoglobulins (Igs)that activate TSH receptor (rTSH). Relapse after withdrawal of antithyroid drugs (ATD) canreach 60%. Measurement of TSH receptor antibodies (TRAb) and thyroid stimulatingimmunoglobulin (TSI) could be an indirect indicator of GD activity. TRAb assays measuresthyroid-stimulating, thyroid-blocking and neutral Igs; TSI assays measures only stimulatingimmunoglobulins. The aim of this study was to evaluate, prospectively, autoimmunity beforeand after ATD therapy for thyrotoxicosis through TSI measurement.**Patients and Methods** Patients were evaluated at the first visit and at the time of ATDwithdrawal. TSH, thyroid hormones, TPO antibody, thyroglobulin antibody, and TRAb weremeasured using eletrochemiluminescent assays Roche Diagnostics; TSI was determined bychemiluminescent assay Siemens Diagnostics. According to manufacturers, TRAb less than 1.75IU/L and TSI less than 0.55 IU/L were negative.**Results** Sixty-seven patients mean age 45,7±2,45 years, 65 women, were evaluated: 50 at thefirst visit, 40 (80%) with GD, and 10 (20%) with toxic multinodular goiter (TMNG). TSIdiagnostic sensitivity (Sen%) and specificity (Spe%) to diagnose GD were 90% and 100%respectively, similar to that of TRAb, of 89% and 100%. Thirty-six patients were evaluated forrecurrence after suspension of ATD (19 of them also had the initial assessment): 21 (58.3%) didnot present recurrence in an mean period of 9.5±2.1 months (3-18); and 15 (41.7%) relapsed in4.4±2.6 months (2-12). The TSI (Sen%) and specificity (Spe%) to predict recurrence were 93%and 38%, respectively; for TRAb they were 67% and 71%. In 10/21 patients who did notrelapse, and whose TRAb was negative, TSI was positive at low levels, which was responsiblefor the low Spe% of this test. Assessing possible other cutoff points for the TSI in therecurrence assessment, an adjustment to 1.4 (TSI less than 1.4 IU/L = negative) raised theSpe% to 86%.**Conclusions** In this group, TSI and TRAb were equivalent for GD diagnosis. For predictingrecurrence, with the proposed cutoff point proposed by the kit manufacturer for TSI a bettersensitivity was obtained, when compared with TRAb (93% versus 67%), despite very lowspecificity (38%); by raising the cutting point to 1.4 specificity could be increased to 86%without reduced sensitivity. A larger sample in needed to support a higher TSI cutoff point inthe clinical routine for the assessment of GD recurrence after ATD.

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Algorithm for the Identification of Hemoglobin Wayne Interference on HbA1c Measurements using Intact Hemoglobin Protein Mass Spectrometry Analysis

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Background: Hemoglobin (Hb) Wayne is a frameshift alpha chain variant that produces two molecules, Hb Wayne I and II. Although clinically silent, the variant causes falsely elevated HbA1c results by HPLC. After a physician reported a discrepantly high HbA1c reported from the D-100 HPLC system (BioRad Laboratories, USA), in which Hb Wayne co-elutes with HbF and HbA1c, we implemented a published algorithm for detecting Hb Wayne variant cases consisting of flagging samples with HbF and HbA1c co-elevations, investigating discordances and using an alternative method for HbA1c testing. We aimed at developing a novel management algorithm for samples with suspected Hb Wayne variant using intact Hb protein mass spectrometry analysis. **Methods:** Samples with elevated HbF >5% and HbA1c >5.7% were

investigated for Hb Wayne interference. When suspicious, samples were evaluated for hemoglobin variants using capillary electrophoresis (CE; Sebia, USA), and Hb intact protein mass spectrometry (MS) analysis and searching for a signature Hb Wayne mass spectra in a rapid assay developed and preliminarily validated in-house (data not shown). In brief, EDTA whole blood was diluted with sample buffer, centrifuged, and the supernatant was diluted and injected into a TLX-II LC system (C18 column, total LC time of 5.5 min) coupled to a Q-Exactive high resolution MS (ThermoFisher Scientific, USA). Mass deconvolution was achieved using Thermo's BioPharma Finder software. **Results:** Five samples were highly suspicious for Hb Wayne from January to October 2019, and were analyzed using the Hb intact protein MS assay. Four of the 5 samples were Hb Wayne positive (HbF range: 6.94-8.48%; HbA1c range: 10.3-12.7%), consistent with Hb variant analysis by CE. **Conclusion:** When feasible, this novel algorithm using Hb intact protein MS analysis is effective in identifying Hb Wayne interference in suspect samples when using the D-100 HPLC system for HbA1c measurement.

Figure 1. Hb Wayne identification workflow using Hb intact protein MS analysis

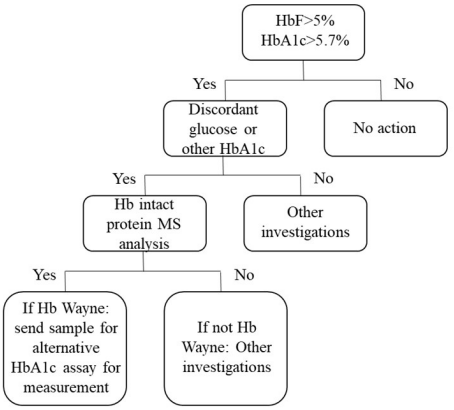

Figure 1. Hb Wayne identification workflow using Hb intact protein MS analysis

A-179

Analytical Performance of HbA1c in 11 Measurement Systems in Colombia

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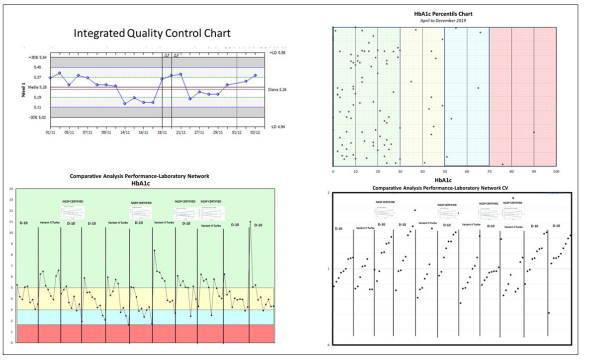
Background: HbA1c is an indicator of diabetes medical care that is used continuously by general practitioners and diabetologists. HbA1c results influence the behavior to be followed with diabetic and prediabetic patients, so the accuracy of the result is essential. A 5% Analytical Total Error is maximum allowed in agree with the American Diabetes Association, ADA to guarantee that the patients results are clinically usefulness Higher measurement errors can negatively affect treatments and behaviors implemented with patients. **Methods:** The performance of 11 HbA1c measurement systems (D-10 and Variant II Turbo) was evaluated over a period of 9 months (April-December, 2019). 4 of these 11 laboratories had NGSP level II certification. These 11 measurement systems verify the performance using integrated quality control charts, (IQCC) with a maximum permissible total error of 5%. The performance was analyzed based on the results of internal quality control with Bio-Rad Lyphocheck Diabetes control, calculating coefficients of variation, biases, analytical errors, sigma metrics and location by percentiles with respect to the performance reported in the Bio-Rad worldwide report corresponding to the months of this study. **Results:** The 11 measurement systems showed imprecisions lower that than 2%, 47 of the 99 measurements lower than 1%. The analytical error of the 99 measurements was lower than 6% in all cases 97/99 lower than 5%. 93 of the 99 measurements were below the 50th percentile, comparing performance with 60 measurement systems worldwide in average. **Conclusion:** The analytical performance of the 11 laboratories was consistent and agreed with the criteria of the NGSP certification, and although only 4 of the 11 had level II certification. The consistent performance of the 11 laboratories were compatible with the NGSP criteria. The adequate use of Integrated quality control charts increase the reliability of HBAa1c results.



A-180

Perfecting Pcats, Developing a More Robust Method for Plasma Catecholamines

A. Senior[1], L. Williams[1], S. J. Marin[2], E. Gairloch[2], C. Desbrow[1], A. Eddington[1], H. Lodder[1], P. Roberts[3]. *[1]Biotage GB Limited, Cardiff, United Kingdom, [2]Biotage LLC, Charlotte, NC, [3]Biotage GB Limited, Charlotte, NC, United Kingdom*

Achieving desired sensitivity of plasma catecholamines by LC-MS/MS can be challenging. Low limits of quantitation can require a highly sensitive MS instrument. Evaporative losses can make concentration of the sample prior to analysis difficult. Removal of matrix components, particularly phospholipids is necessary for maximum sensitivity and accuracy. This study was conducted to optimize conditions to improve recovery and reduce ion suppression for analysis of plasma catecholamines.

Plasma was obtained from bioIVT (Burgess Hill, UK). Standards were purchased from Sigma-Aldrich (Dorset, UK). Solvents and other reagents were purchased from Rathburn (Walkerburn, UK), Honeywell (Bucharest, Romania) and Sigma-Aldrich. Solid phase extraction (SPE) was performed using EVOLUTE EXPRESS 10 mg WCX (Biotage Cardiff, UK). Multiple sample pretreatments, organic washes and elution solvents were evaluated. Replicate samples (n=7) were prepared and extracted. Samples were analyzed using a Nexera UHPLC coupled to a Sciex Triple Quad 5500. Several LC columns were evaluated: ACE Excel 1.7 3.0 x 100 mm 1.7 dp and ACE Excel C18 2.1 x 100 mm 1.7 dp (VWR, Lutterworth UK); Raptor Biphenyl 2.1 x 100 mm 2.7 dp (Thames RESTEK, Saunderton UK); Acquity HSS T3 C18 2.1 x 100 mm 1.9 dp (Waters, Elstree UK).

Analyte functionality resulted in sample pre-treatment and aqueous wash optimized to a pH of around 6. Further washing with combinations of organic solvents such as methanol, acetonitrile and 2-propanol were evaluated for best baseline and signal to noise. An additional wash of dichloromethane proved to reduce phospholipids in the extracted sample. Large differences were observed in interference profiles when investigating matrix anticoagulant. Final wash protocol was dependent on matrix anticoagulant. Elution protocols were evaluated for: elimination of evaporation using 85:15 water:2-propanol with 0.1% formic acid with direct low volume LC-MS/MS injection, and incorporation of an evaporation step to allow a concentration factor. Here elution was performed with 80:20 MeOH:water with 2% formic acid followed by reconstitution in up to 200 uL of 5:95 MeOH:water for LCMS/MS analysis. No evaporative losses were observed at 37°C. Option 1 demonstrates lower phospholipid residues, option 2 provides further opportunity for analyte concentration.

Column geometry was an important factor for chromatographic performance due to organic content in the elution solvent. The final LC method used an ACE Excel C18-PFP 1.7 um, 3.0 x 100 mm column with Restek Raptor ARC18 guard in Optimize-EXP format at 0.4 mL/min. Gradient separation using 0.5 mM ammonium fluoride and methanol separated the compounds of interest.

Linearity was verified between 0.04 and 1.28 ng/mL for norepinephrine and dopamine and between 0.02 and 1.28 ng/mL for epinephrine, normetanephrine, metanephrine and 3-methoxytyramine. Accuracy and imprecision was within 10%. Recoveries ranged from 65-90%.

Pretreatment with 0.05% formic acid and SPE provided clean samples for LC-MS/MS analysis. 2-propanol and dichloromethane washes proved superior for phospholipid removal. Elution without evaporation is feasible with a low volume injection using a 3 mm column. Evaporation without analyte loss permitted larger injection volumes of more concentrated samples for maximum sensitivity.

A-181

Bone Biomarkers Predict High Turnover vs. Low Turnover Osteoporosis

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Background: Assessment of bone turnover status in osteoporosis is important for therapeutic decision making. While osteoporosis is diagnosed by bone mineral density scanning, bone biopsy is currently the gold-standard method for categorizing high turnover (HTO) versus low turnover osteoporosis (LTO). However, bone biopsies are invasive and associated with complications. A variety of biomarkers are utilized to monitor changes in bone formation (e.g. bone-specific alkaline phosphatase (BSAP) and osteocalcin) and resorption (e.g. N and C-telopeptide) over time to monitor therapy, but these are often influenced by preanalytical variables which limit their clinical utility. To date, no single biomarker has sufficient diagnostic strength to predict bone biopsy results. A combination of biomarkers may accurately predict bone turnover rates in osteoporosis, thus providing a less invasive alternative to the bone biopsy. **Objective:** To assess the diagnostic utility of individual and bone biomarker panels to predict the rate of bone turnover in patients with osteoporosis.

Methods: Adult patients attending the University of Kentucky Nephrology, Bone and Mineral clinic with a diagnosis of osteoporosis and a bone biopsy performed between 2016 and 2019 were included in the study. Patients were excluded if they had chronic kidney disease, insufficient biopsy samples, or no bone biomarkers measured up to a year before or 60 days after biopsy. Electronic medical records were reviewed for patients' diagnoses, biopsy results, demographics, and bone biomarker (calcium, phosphorus, creatinine, BSAP, 25-hydroxyvitamin D, N-telopeptide, C-telopeptide, parathyroid hormone, osteocalcin, pyridinium crosslinks) results. Descriptive statistics and ROC analysis of bone biomarker concentrations in osteoporotic patients was performed using GraphPad Prism version 8.3.1. Multi-marker models to predict LTO and HTO were developed as follows: Stratified 10-fold cross-validation assessed the accuracy of eight supervised machine learning algorithms. The classifier with highest accuracy score was used to build a final model which was first trained on the training dataset and performance was evaluated against a test dataset. The machine learning and data processing were carried out in Python version 3.5.2 with included modules Pandas version 0.18.1 and Scikit-learn version 0.18.1. **Results:** A total of 76 patients, 33 LTO, 32 HTO, and 11 with mild or normal turnover osteoporosis were included. When evaluated alone, only osteocalcin and N-telopeptide demonstrated significantly different median concentrations between LTO and HTO osteoporosis patients ($p < 0.05$ for both). Areas under the ROC curve for Osteocalcin and N-Telopeptide to differentiate HTO from other osteoporotic patients were 0.67 and 0.73, respectively. Out of the eight machine learning algorithms, a predictive model based on the linear Support Vector Machines (SVM) was developed. The optimized model yielded an accuracy score of 82% when applied to test dataset for prediction of LTO verus HTO. Combined, the biomarkers more efficiently predicted HTO versus LTO than any marker alone.

Conclusion: A model consisting of 9 biomarkers associated with bone physiology more efficiently predicted HTO versus LTO. Future studies will investigate biomarker panel changes over time in larger populations of patients to determine if change correlates with biopsy and predicts response to treatment.

A-182

Prevalence of Hypovitaminosis D in Infants in a Big Database of a Laboratory Network of Clinical Analysis in Brazil

L. L. A. Cavalcante[1], M. C. G. Castelo[1], S. P. Bandeira[1], R. G. Fontes[2], C. d. W. Sabino[3], N. P. D. Linhares[1], F. P. de Oliveira[3], R. F. B. Canineu[3], L. C. Vieira[3], G. A. Campana[3]. *[1]DASA, Fortaleza, Brazil, [2]DASA, Rio de Janeiro, Brazil, [3]DASA, São Paulo, Brazil*

Background: Vitamin D (VD) is essential for normal human development since intrauterine life. Epidemiological studies show that VD deficiency has a high prevalence, even in those countries with adequate sunlight for most of the year. In Brazil, the Brazilian Pediatric Society determine vitamin D supplementation from birth to 2 years of age. The assessment of vitamin D is recommended for groups at risk. **Objective:** To analyze the requested of 25- hydroxyvitamin D (25(OH)D) results for children (0 to 2 years old) in a network of laboratories in Brazil. **Method:** A cross-sectional study was conducted to evaluate all patients with measurement of 25(OH)D performed in the largest network of clinical analysis laboratories in Brazil, between January 2016 to December 2019. Plasma VD concentration was determined by electrochemiluminescence immunoassay performed using ADVIA Centaur XP platform Siemens. In individuals with more than one mesurement of the VD, the lowest value was considered. The patients were divided into categories by sex, age and in serum levels of VD. The subjects were characterized as having low VD according to the criteria of the Official Positioning of Brazilian Society of Clinical pathology/Laboratory Medicine and the Brazilian Society of Endocrinology and Metabolism (2018): < 20 ng/mL (<50 nmol/L) for healthy population (up to 60 years) and < 30 ng/mL (< 75 nmol/L) for at-risk groups such as infants. Serum levels of vitamin D were described in mean and standard deviation. **Results:** 8,497,346 samples were analyzed. Of this total, 143,163 were infants (1.68%), 50.5% females. The mean VD in this group was 38.37 ± 14.69 ng/mL. When using the cutoff point of 30 ng/mL (<75 nmol/L), 30% of the samples showed lower values than this; and using the cutoff point of 20 ng/mL (<50 nmol/L), 6.4% were deficient in VD. **Conclusion:** Our findings show that the mean 25OHD in this population is in level of sufficiency. However, a significant percentage is below the recommended value. Due to the use of databases, definitive conclusions cannot be made in the interest of more complete data such as the presence of risk factors for hypovitaminosis D, use of interfering drugs or the absence of the recommended supplementation.

A-184

Comparison of GH Performance after Clonidine: Morning Versus Afternoon

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Background: Even after validation of fasting flexibility for most of routine exams, laboratories still tend to be overcrowded in the morning and empty in the afternoon. Traditionally, GH stimulus tests are performed in the morning, further burdening the laboratory routine. So, we hypothesized whether performing collection of GH simulation test with Clonidine in the afternoon could be feasible without resulting in impaired response to the secretagogue.

Methods: We performed a retrospective analysis of children submitted to the GH stimulation test with Clonidine in a laboratory reference center over a period of 6 month. Children were divided into 2 groups: *Group 1* (*patients submitted to GH sampling in the afternoon*) and *Group 2* (*patients submitted to GH sampling in the morning*). Children who were submitted to the test in the afternoon were instructed to a 4 hours fasting time prior to the test and children submitted to the test in the morning were instructed to a fasting time of 8 hours.

Results: Thirty six patients were submitted to GH sampling after stimuli with Clonidine at the afternoon (group 1) and 82 patients were submitted to the test in the morning (group 2) . Thirty two (89 %) of patients in group 1 were responsive to the stimulus versus 70 (85 %) patients in group 2, which was not statistically significant ($p = 0.7$).

Table 1- Comparison between mean peak GH response in Group 1 (blood sampling in the afternoon) and Group 2 (blood sampling in the morning)

Time	GROUP 1 (n=36)	GROUP 2 (n=82)	p
GH - Baseline (ng/mL)	2.5	1.18	0.012
GH - 60 minutes (ng/mL)	8.3	7.3	0.70
GH - 90 minutes (ng/mL)	11	7.5	0.08

Conclusion: Although our sample is still small, we conclude that submitting patients to GH stimulation test with Clonidine in the afternoon does not seem to compromise the response of the test. This procedure helps reducing the overload of laboratory assistance in the morning and allows making the patient's experience in the laboratory a little bit more pleasant.

A-185

Serum 25- Hydroxyvitamin D Status in Elderly in a Big Database of a Laboratory Network of Clinical Analysis in Brazil

L. L. A. A. Cavalcante[1], M. C. G. Castelo[1], S. P. Bandeira[1], C. d. W. Sabino[2], R. G. Fontes[3], F. P. de Oliveira[2], R. F. B. Canineu[2], L. C. Vieira[2], G. A. Campana[2]. *[1]DASA, Fortaleza, Brazil, [2]DASA, São Paulo, Brazil, [3]DASA, Rio de Janeiro, Brazil*

Background: Epidemiological studies have shown that vitamin D (VD) deficiency has a high prevalence in elderly population, even in regions with high sun exposure. Low levels of vitamin D are associated with impaired bone health, increased risk of falls and fragility fractures. **Objective:** To analyze the requested of 25-hydroxyvitamin D (25 (OH) D) results in the elderly in a network of laboratories in Brazil.

Method: A cross-sectional study was conducted to evaluate all patients with measurement of 25(OH)D performed in the largest network of clinical analysis laboratories in Brazil, between January 2016 to December 2019. Plasma VD concentration was determined by electrochemiluminescence immunoassay performed using ADVIA Centaur XP platform Siemens. In individuals with more than one mesurement of the VD, the lowest value was considered. The patients were divided into categories by sex, age and in serum levels of VD. The subjects were characterized as having low VD according to the criteria of the Official Positioning of Brazilian Society of Clinical pathology/Laboratory Medicine and the Brazilian Society of Endocrinology and Metabolism (2018): < 20 ng/mL (<50 nmol/L) for healthy population (up to 60 years) and < 30 ng/mL (< 75 nmol/L) for at-risk groups such as infants. Serum levels of vitamin D were described in mean and standard deviation. **Results:** 8,497,346 samples were analyzed. Of this total, 2,100,659 were elderly (24,7%), 72,2% female. The mean VD in this group was 28,6 ± 11,9 ng/mL, smaller in female (27,5 ± 11,2 ng/mL). When using the cutoff point of 30 ng / mL (<75 nmol / L), more than half the samples showed lower values than this (63.3%); and using the cutoff point of 20 ng / mL (<50 nmol / L), 21.0% were deficient in VD. Conclusion: Our findings show that more than half of the population over the age of 60 is at inadequate levels according to current recommendations. Definitive conclusions cannot be made in the interest of more complete data such as medication use, degree of sun exposure, presence of diseases, among other factors that may interfere with vitamin D status.

A-186

Serum TSH Status in the Elderly Population in a Database of a Laboratory Network of Clinical Analysis

L. L. A. Cavalcante[1], M. C. G. Castelo[1], S. P. Bandeira[1], R. G. Fontes[2], C. d. W. Sabino[3], G. A. Campana[3], F. B. Furtado[1]. *[1]DASA, Fortaleza, Brazil, [2]DASA, Rio de Janeiro, Brazil, [3]DASA, São Paulo, Brazil*

Background: Thyroid function is regulated by a dynamic hormonal system. In clinical practice, TSH is the recommended test for the diagnostic screening of thyroid function due to its high sensitivity, especially by third generation electrochemiluminescence immunoassay. Objective: To determine the TSH serum status in elderly population in a clinical analysis laboratory network in Fortaleza, Brazil. Method: A cross-sectional study was conducted to evaluate all patients over 60 with measurement TSH performed in a clinical analysis laboratory network in Fortaleza, Brazil, between January 2019 to December 2019. Plasma TSH concentration was determined by electrochemiluminescence immunoassay performed using ADVIA Centaur XP Siemens platform. The patients were divided into categories by sex, age (61 to 80 years and above 80 years) and in serum levels of TSH. The reference value established was that defined by the test kit used, as follows: 0.4 to 5.8 µIU / L for the age group from 61 to 80 years and 0.4 to 6.7 µIU / L for over 80 years. Serum levels of TSH were described in mean and standard deviation. Results: 105,564 samples were analyzed. Of this total, 75% female. The mean TSH total group was 2.53 ± 7.19 µIU / L. In the group aged 61 to 80 years, the mean was 3.42 ± 6.90 µIU / L (71.9% female) and 2.43 ± 4.55 µIU / L in group above 80 years (73.6% female). 5.2% of individuals aged 61 to 80 years had TSH lower than the lower limit of the applied reference value (83.6% female) and 5.7% higher than the upper limit of normal (72.7% female). In the group over 80 years old, 4.0% had TSH lower than the lower limit of the applied reference value (85.7% female) and 6.3% higher than the upper limit of normal (69.2% female) . Discussion: The TSH concentrations could be influenced by several conditions. Observational studies report that elevated TSH returns to normal in 37% to 62% of patients without treatment and in 51% of cases in which low TSH is found. It is recommended to perform a new TSH measurement with correlated exams for diagnostic clarification. Conclusion: A small percentage of elderly people had TSH values outside the reference range. Due to the use of databases, interfering factors including iodine nutritional status, presence of thyroid autoantibodies, thyroid disease, medication, nonthyroidal illness and assay type. cannot be analyzed and definitive conclusions cannot be made.

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Genomics/Genetics

A-187

Performance Evaluation of Multiclass Neural Network for Clinical Variant Interpretation

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Background: Accurate variant classification and identification of a pathogenic variant are essential for medical decision such as tailored therapy as well as risk stratification and diagnosis of diseases. However, our understanding on classification and interpretation of variants is far from being perfect. Here, we evaluated the performance of neural network using multiple databases for variant interpretation and the importance of database selection.

Methods: The pathogenicity of a total of 32,605 variants registered in HGMD were predicted using multiclass neural network. A total of 25 in-silico predictors and databases were used as features and the annotations from ClinVar [Pathogenic/Likely Pathogenic (P/LP, n=20,840), Variant of unknown significance (VUS, n=10,725), Benign/Likely benign (B/LB, n=1,040)] and InterVar (P/LP (n=12,031), VUS (n=19,026), B/LB (n=1,548) were used as training labels. We used Microsoft Azure machine learning studio to collect statistics about accuracy.

Results: When neural network was trained using ClinVar annotations, the overall accuracy was 0.95 (average accuracy of 0.96). On the other hand, when trained based on InterVar classification, the overall accuracy was 0.90 (average accuracy of 0.96). There were no major categorical changes regardless of the training labels. The differences in the overall accuracy of neural network were attributed to the differences in predicted class of VUS.

Conclusion: This study demonstrates that the differences of the training database could result in the differences of VUS interpretation. In addition, we showed that annotation discordances between public databases could be overcome by use of the application of neural network. This study suggests that application of neural network could be an accessible alternative when clinically interpreting the pathogenicity of VUS registered in public database.

A-189

Establishment of Next-Generation Sequencing Based Polydactyly Gene Panel

J. Hong, K. Park, H. Kim, W. Lee, S. Chun, W. Min. *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea, Republic of*

Background: Polydactyly is a congenital condition characterized by extra digits, though its phenotype varies from skin tag to complex duplication. Polydactyly is an anomaly observed right after birth, and it can occur either alone or as a sign of various syndromes. The main etiology of polydactyly is known to be genetic. However, genetic testing for polydactyly is not routinely performed. We established a gene panel for polydactyly using next-generation sequencing (NGS), a method that can analyze many genes simultaneously.

Methods: We reviewed literatures and searched databases such as Human Gene Mutation Database and Online Mendelian Inheritance in Man to list up and select genes associated with polydactyly. BED file including exons and part of introns of selected genes was created and probes were designed. The reagents used were DxSeq Library Preparation Reagent and DxSeq Target Capture Reagent. Illumina Miseq DX was used for sequencing. Bioinformatics software employed were Illumina VariantStudio and DxSeq Gene Analysis System. Reporting methods followed standards and guidelines of the American College of Medical Genetics and Genomics. Designed panel was applied to NA12878 reference genome to validate and the result was compared to databases such as Genetic Testing Reference Materials Coordination Program and The Genome in a Bottle Consortium hosted by NIST.

Results: Overall, 167 genes and ZRS, a region in intron of LMBR1 gene, were included in the polydactyly panel. 503,063 base pairs of target region were assigned. Several regions that repeatedly generate low coverage due to deletion and GC bias were excluded from target and left to manual review and Sanger sequencing if needed. Sequencing and analyzing of NA12878 were performed according to the established panel and protocol. 205 previously reported variants in the target region were detected in all duplicated samples of NA12878, yielding analytical sensitivity of 100%. The match rate was calculated for the detected sequence and the high-confidence region of NIST, and the analytical specificity and accuracy were over 99.9%.

Conclusion: To test many genes related to polydactyly at once, NGS is a suitable method. Some regions showed low coverage and made QC workflow difficult. We excluded the regions from target and prepared manual review process and Sanger sequencing to solve the problem. In conclusion, polydactyly NGS panel can be used for detecting causal genes of polydactyly and benefit patient management.

A-190

Alpha-1-Antitrypsin Molecular Testing in Canada: A Seven Year, Multi-Centre Comparison

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Background: Laboratory confirmation of the diagnosis of α_1-antitrypsin (A1AT) deficiency may be achieved by multiple methods, which may be combined into various algorithms. The goal is to optimize the diagnostic process maximizing both sensitivity, and specificity. Molecular confirmation further provides patients with the information needed for accurate genetic counselling in the context of early diagnosis and prevention for their relatives and offspring. In this study, we sought to compare the relative comprehensiveness of pathogenic variant detection of the different protocols utilized in four major diagnostic centres in Canada. From that we aimed to identify an optimal strategy to efficiently maximize the number of diagnoses of pathogenic variants.

Methods: Diagnostic test results from 2011 to 2018 at clinical laboratories in British Columbia (BC), Alberta (AB), Ontario (ON), and Québec (QC) were retrieved and reviewed. The four labs utilize the following protocols: BC-CGID (serum A1AT Concentration/Genotyping/Isoelectric focussing (IEF)/DNA sequencing), AB-CID (serum A1AT Concentration/IEF/DNA sequencing), ON-CD (serum A1AT Concentration/DNA sequencing), and QC-G (Genotyping). As the respective catchment areas varied in size and ethnic composition, the comprehensiveness of pathogenic variant detection was assessed by comparing the frequency of individual genotypes to the ZZ genotype, which is clearly identified by all protocols.

Results: Across the four laboratories 5399 index patients were tested, and 396 ZZ genotypes were detected. The preliminary serum A1AT concentration screen proved to be an extremely useful method to identify patients for further testing. Serum screen followed by sequencing (ON-CD) had the highest detection rate for pathogenic variants. With this algorithm, genotypes with at least one pathogenic deficiency variant, other than S, Z or F, were identified at a rate of 0.67 /ZZ genotype as compared to < 0.2 /ZZ using the other algorithms. While the ON-CD approach

was most sensitive it also had the highest rates of genotypes containing an undefined molecular variant (UMV) (0.16 /ZZ), and genotypes containing a likely benign variant (0.08 / ZZ), as compared to the other methods (<0.12 /ZZ and < 0.06 /ZZ, respectively). The F variant was identified within a compound heterozygote genotype at a rate of 0.10 /ZZ, only in the ON-CD and the AB-CID protocols. Across all four centres, M Malton was the next most common variant, identified as a compound heterozygous genotype at a rate of 0.04 /ZZ, only in the ON-CD and BC-CGID protocols.

Conclusion: A1AT deficiency testing protocols vary in the efficiency of detection of rare pathogenic variants. As anticipated, strategies which more readily detect pathogenic variants also are more likely to detect UMV and likely benign variants. This comparative data will be useful to laboratories planning an A1AT diagnostic service.

A-191

A Case of Congenital Neuroblastoma Associated with Noonan Syndrome

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Background: A 35-year-old pregnant woman was evaluated by routine prenatal ultrasound at 12 weeks of gestation, and an increased nuchal translucency was observed. Thus, a chorionic villus sample was obtained to perform genetic studies. **Methods:** For chromosome analysis, G-banding Karyotyping and fluorescent *in situ* hybridization were performed on chorionic villus biopsy. PCR and direct sequencing of PTPN11 exons 2, 3, 4, 7, 8 12, 13 were carried out on both chorionic villi and amniotic fluid in order to identify a possible causal mutation of Noonan Syndrome (NS). Metanephrine, normetanephrine, vanillylmandelic acid and homovanillic acid were determined on single-voided urine samples collected during 3 consecutive days by high-performance liquid chromatography (HPLC) using the Agilent® 1260 Infinity analyzer (Agilent Technologies). Finally, urinary creatinine was quantified in the ADVIA® Chemistry XPT System (Siemens Healthineers). **Results:** The chorionic villus biopsy revealed a normal fetal karyotype 46XX. Because of the abnormal ultrasound findings, we expanded the study to rule out a rasopathy. The heterozygous mutation N308D (c.922 A>G, p.Asn308Asp) was found in exon 8 of the *PTPN11* gene in both chorionic villi and amniotic fluid. The mutation was proven to be *de novo*, and the parents were counselled about the implications of the result. After discarding any further malformation, the parents decided to continue the pregnancy and an asymptomatic female infant was delivered at term (39+3 weeks). In the context of NS- associated screening, an adrenal mass of 2.1 x 2.7 cm was found. The analysis of the 3 urine samples revealed a high Normetanephrine/Creatinine ratio in all of them: 5361.3, 2818.8 and 11796.7 (Reference Value 0-3 months: 590-1520 nmol/mmol creatinine), so the patient was diagnosed with Neuroblastoma. **Conclusion**: NS is an autosomal dominant condition with an incidence of 1:1000-2500 live births. It is characterized by short stature, typical craniofacial dysmorphism, congenital heart defects, variable developmental delay and predisposition to malignant tumors. From a molecular point of view, mutations in the *PTPN11* gene are identified in approximately 50% of patients with NS**.** This gene encodes the SHP-2 protein tyrosine phosphatase, which play an important role in several intracellular signal transduction. Thus, gain of function mutations can result in malignant transformation by intracellular accumulation of tyrosine-phosphorylated proteins. On the other hand, neuroblastoma (NBL) is a neural crest derived malignancy of the peripheral nervous system affecting 1:100000 live births and represents 6%-10% of all childhood tumors. Measurement of secreted catecholamine metabolites, imaging techniques and genetic analysis of tumor DNA are used for diagnosis in patients with NBL. Although the association between NBL and NS is rare, this case illustrates the need to contemplate all the possible clinical outcomes associated with *PTPN11* mutations, especially in the prenatally diagnosed cases.

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Comparison of Different Molecular Assays to Diagnose Influenza A Virus, Influenza B Virus and RSV Infection

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Background: Influenza virus and respiratory syncytial virus (RSV) are a significant cause of morbidity and mortality worldwide. Although its diagnosis is often made by clinical signs and symptoms, laboratory testing may be needed to guide antiviral therapy, determine isolation precautions, and provide epidemiologic data, since many different respiratory viruses can cause influenza-like illness. The laboratory diagnosis of influenza has evolved from the use of antigen detection tests to molecular methods that are now considered the new gold standard. This work focused on evaluating the efficacy of the Viasure *Flu A, Flu B & RSV* real time PCR product for their diagnostic application in clinical laboratories. The sensitivity and specificity of the assay was compared with the hospital routine method.

Methods: 103 Nasopharyngeal swabs from patients with flu-like symptoms were collected during 2018-2019 flu-season and were tested in parallel with both methods. The routine analysis was performed using Cobas® Influenza A/B & RSV nucleic acid test. For Viasure analysis RNA purification was performed with the automated extraction system PSS MagLEAD12gC. The evaluated method was used according to the manufacturer's instructions in DTlite system (DNA-technology)**.**

Results: Viasure assay detected the presence of Flu A in 22 samples, whereas the reference method detected 23 positive specimens. The sensitivity and specifity of this test for this pathogen was 91% and 98% respectively. Concerning RSV detection, the tested method identified this pathogen in 15 samples, one of them was not identified with the Cobas® Liat. In the same way, Cobas system detected the presence of RSV in 15 samples, one of these samples was not detected with Viasure method. The sensitivity and specificity values obtained for RSV were 93% and 98% respectively. None of these assays detected Flu B positive specimens.

Conclusion: Evaluated assay provides highly sensitive, specific and rapid detection of Flu A, Flu B and RSV being a good alternative to other diagnostic techniques. During Flu season a hospital receives dozens of samples with Flu clinical suspicion every day. Viasure allows in a single run the analysis of 96 samples giving the diagnosis to the patients in 2 hours.

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Diagnostic Performance and Validation of Two Molecular Methods for the Detection of VRE Infection

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Background:

Infections due to vancomycin resistant *Enterococci* (VRE) are a problem worldwide and are mostly mediated by *vanA* and *vanB* genes. Recognition of colonized patients represents a crucial step in controlling the dissemination of these organisms and is a laboratory-based strategy. Culture of rectal swabs is traditionally used to identify VRE colonized individuals and the turnaround time of this test typically exceeds 48h. The use of protocols that include molecular methods may contribute to reduce the time necessary to obtain results, thus providing the possibility of an intervention in a more convenient timeframe.

This work focused on evaluating two molecular methods and compared their characteristics with our routine diagnostic method.

Methods:

A comparative study was performed with 216 faecal clinical samples collected from June to August 2019 of suspected VRE infection patients attended in Western Sydney Local Health District (WSLHD). Routine diagnosis consisted of an in-house real-time PCR adapted to the BD MAX™ platform, followed by culturing onto VRE chromogenic agar medium of the positive PCR cases. The evaluated molecular methods were Viasure *Vancomycin resistance* Real Time PCR Detection Kit for BD MAX™ system and the same product in open format. Both detect *vanA* and *vanB* genes and are presented in lyophilized format. The evaluated methods were performed according to their manufacturer's instructions.

Results:

Routine analysis showed that *vanA* gene was present in 65/216 samples, 35/216 were positive for *vanB* gene and in 17/216 samples both genes were observed. No vanA or vanB genes were found in 99/216 samples.

The commercial kit for BD MAX™ system showed 65/216 samples positive to *vanA* gene, 36/216 samples positive to *vanB* gene and in 17/216 samples both genes were observed. No *vanA* or *vanB* genes were found in 98/216 samples. Therefore, the sensitivity and specificity values calculated with a 95% confidence interval were >99% for the vancomycin resistance related genes using this kit.

In addition, the commercial kit in open format showed 64/216 samples positive to *vanA* gene, 36/216 samples positive to *vanB* gene and in 16/216 samples both genes were observed. No *vanA* or *vanB* genes were found in 100/216 samples. Therefore, the sensitivity and specificity values calculated with a 95% confidence interval were >94% for the vancomycin resistance related genes using this kit.

Conclusion:

Evaluated methods provided an excellent correlation with the routine analysis and therefore, demonstrated to be good tools for the diagnostic of VRE. A highlight feature of both products was their lyophilized format. The manipulation was minimal, without intermediate mixtures, and was useful for storage at room temperature, instead of using refrigerated containers and occupying place in the freezers of the hospital.

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VA Precision Oncology Program; A Tampa Success Story

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Background: The Veteran's Administration's (VA) National Precision Oncology Program (NPOP) aims to provide targeted cancer care for Veterans, based on their tumor genetic profiles, and facilitate their access to new investigational therapies through clinical trials. Molecular testing is recommended to identify rare driver mutations that may be present in tumor tissues that would have an impact on treatment. Proven responses to molecular therapies have greatly increased the improvement of care in cancer patients. The objective of the study was to evaluate effectiveness of the NPOP at the Tampa Veteran's Hospital. **Method:** Data for a twelve-month period (1/1/2019-12/31/2019) was extracted from our computerized patient record system (CPRS) and analyzed using an MS Excel program. **Results:** A total of 83 patient's tumors were submitted for next gen sequencing. 73% of cases have at least one actionable alteration. The average number of alterations per sample was 6, and the average number of therapies with clinical benefits per sample was 6. The average number of clinical trials per sample was 14. The total number of therapies with clinical benefit based on the molecular results was 178. Total number of available therapies with clinical benefit in tumors other than the patient's tumor type was 307. 2 samples show high tumor mutational burden as defined by 16 or more mutations per sample. Cancers of lung and colon accounted for 40% of tumor samples sequenced. **Conclusions:** Next generation sequencing provides accurate and comprehensive data detailing actionable gene alterations within a tumor sample. Tumor molecular alterations are listed in categories including: consequence of sequence mutation, exon location, and fold increases for amplifications. Gene alterations are further designated by current FDA approved therapy (for site specific indications and indications in similar tumors in other sites) and therapies currently in clinical trials. Based on our findings, we conclude that the VA's National Precision Oncology Program is a total success and provided the majority of patients with the most current and advanced therapeutic options.This material is the result of work supported with resources and the use of facilities at the James A. Haley VA Hospital.

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Clinical Impact of Pharmacogenomic Testing

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Background: Adverse drug reactions are estimated to cost upwards of $136 billion per year and lead to >100,000 deaths annually. A patient's genetic background is a large determinant of his or her response to drug and pre-disposition to side effects. The goal of pharmacogenomics is to enable providers to deliver the right drug, at the right dose, to the right patient. This may be accomplished by characterizing pharmacologically-relevant genes using DNA extracted from whole-blood to identify single-nucleotide variants known to affect the function of the encoded protein. Most significant is the Cytochrome P450 (CYP) isoenzyme superfamily, whose members are responsible for the metabolism of approximately 75% of drugs. The genes for these enzymes are subject to various degrees of polymorphism, particularly CYP2D6, which has approximately 40 known variants. The genetic variation in this and other cytochrome enzymes often leads to phenotypical variation and can result in variable response to many drugs. **Method:** To assess the clinical impact of routine pharmacogenomic profiling, randomly selected remnant whole blood samples were subjected to DNA extraction and genotyping of relevant SNVs by mass spectrometry using a customized Agena PGX74 assay. Translational SoftwareTM was used to predict likely response to a variety of frequently prescribed therapeutic compounds using the genotypic results for each specimen. **Results:** Complete genotypic and phenotypic calls for CYP isoenzymes 1A2, 2B6, 2C19, 2C9, 2D6, 3A4, and 3A5 were obtained from 150 samples. An additional 88 samples gave partial genotyping calls. Out of 237 samples, 164 had genetic variants that would necessitate an increased or decreased dose for at least one drug. Of those, 61 samples would require dose adjustment for 2-9 drugs, 25 samples would require dose adjustment for 10-29 drugs, and 27 would require dose adjustment for 30 or more drugs. **Discussion and Conclusions:** Due to study design, more impaired enzyme activity was observed than increased metabolic rate which often leads to increased exposure to the parent compound. The extent of risk for each patient:drug combination depends on three main factors: the degree of predicted enzyme impairment or activation, the width of the therapeutic window of the drug, and whether the parent compound or metabolite is the active molecule. This results in metabolic variations which can range from significant risk of toxicity to frank failure of the drug to provide any therapeutic value. Pharmacogenomic profiling can aid in reducing the potential for adverse drug reactions by allowing up-front selection of an appropriate dose to fit the patient's unique metabolic profile.

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DNA Origami Nanoprobe for Direct Visualization of Extracellular Vesicle microRNA In Situ

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Background: Exosome hold paramount importance in mediating intercellular communication and direct tracing of exosomal inclusions holds unprecedented importance for elucidating the mechanisms. However, High-fidelity determination of exosomal molecular markers *in situ* has yet to be achieved due to the difficulty of transporting molecular probes into intact exosomes.

Methods: We deliberately designed a DNA tetrahedron structure to ensure a minimal size for better penetration whilst keeping a rigid geometry. We then invented a tetrahedron nanoprobe for *in situ* tracing of exosomal miRNA cargo. A lung cancer model was employed to evaluate the construction of the proposed tetrahedron nanoprobe as it is the leading cause of cancer-related deaths worldwide. After checking the feasibility of the proposed nanoprobe for high-fidelity tracing exosomal miR-21 cargo, we also evaluated its *in vitro* diagnostic potential by comparing the results of clinical samples with RT-qPCR and digital PCR (ddPCR).

Results: We designed a tetrahedron scaffold with three fluorophore donors at the terminal of each vertex in the bottom plane, and the hairpin probe was terminated by a quencher; In the presence of specific miRNAs, the stem-loop structure would be damaged and fluorescence turn-on could be realized. Additionally, a target concentration-dependent FI increase with a good linearity in the range from 1 pM to 10 nM and a detection limit of 45.4 fM. The penetrating capability of the proposed DNA origami nanoprobe was validated by delivering the nanoprobe into both A549 cells and exosomes. We utilized the nanoprobe to test the artificially modulated exosomal miR-21, and the results indicated that both the proposed probe and ddPCR produced identical results for those upregulated or downregulated samples, further validating the feasibility of the nanoprobe for detecting exosomal miR-21 at different concentrations. Furtherly, the nanoprobe and the RT-qPCR method could differentiate non-small cell lung cancer patients from healthy persons.

Conclusion: We describe a robust and reliable tetrahedron nanoprobe for direct visualization of exosomal miRNA *in situ* without the need for damaging the exosome membrane or extracting exosomal inclusions. By employing a DNA nanotetrahedral scaffold to facilitate exosome penetration, a hairpin probe can be conveniently delivered into the exosome to enable real-time detection of various exosomal inclusions. This technology opens an avenue for tracing exosomal inclusions *in situ*, providing a novel strategy to investigate exosome-derived fundamental biological mechanisms and explore subsequent clinical applications.

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New Alternatives for the Simultaneous Detection of Several Pathogens involved in Sexually Transmitted Diseases (STIs)

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Background: Despite the growing concern about the impact of STI in public health, several of these diseases still represent major epidemics. As STIs are often asymptomatic, their clinical identification is difficult. Molecular methods offer increased sensitivity and specificity. They are useful for pathogens that cannot be cultivated and help diagnose atypical cases, asymptomatic or multiple infections. We could evaluate a new ready-to-use multiplex real-time PCR assay for the detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), *Ureaplasma parvum* (UP), *Ureaplasma urealyticum* (UU), *Mycoplasma genitalium* (MG) and *Mycoplasma hominis* (MH) in clinical specimens.

Methods: A prospective evaluation was performed using 948 clinical samples. The clinical samples included were: first-void urines and rectal, urethral, endocervical, vaginal and pharyngeal swabs. The bacterial nucleic acid from fresh and frozen clinical samples was extracted using STARMag 96x4 Universal Cartridge Kit (Seegene, Korea) into NIMBUS or Microlab STAR Let automatic extraction system (Hamilton, Switzerland) according to the manufacturer's instructions. After that, in parallel, DNAs were directly used for real time PCR analysis using Allplex™ STI Essential

assay (Seegene, Korea) in CFX96™ Real-time PCR system (Bio-Rad) and VIASURE *Sexually transmitted diseases* Real time PCR kit (CerTest Biotec, Spain) in DTlite Real-Time PCR System (DNA Technology).When a discrepant result was obtained, the sample was again analysed with FTD Urethritis plus (Fast Track Diagnostics).

Results: A total of 948 samples were analysed. 407/948 were at least positive for one of the seven pathogens. Both assays totally agreed for 902 (95.14%) specimens tested. Discordant results were obtained in 46 samples. These discrepancies were mainly observed in samples close to the limit of detection of both kits. Therefore, positive results with CT value obtained from 35 and low amplification signal should be applied with caution and should be interpreted based on the patient´s clinical data. In 35 of the discordant samples, it was possible to solve the discrepancy using FTD Urethritis plus (Fast Track Diagnostics). The rest could not be re-analysed for lack of sample. It was observed that this third kit confirmed results obtained by both products, not observing a confirmation tendency of only one of the two kits. The concordance was 99.47% for CT (κ=0.96), 99.68% for MG (κ=0.94), 99.78% for NG (κ=0.94), 99.68% for TV (κ=0.92), 98.62% for UU (κ=0.95), 98.10% for UP (κ=0.96) and 99.78% for MH (κ=0.99). The sensitivity of VIASURE *Sexually Transmitted Diseases* Real Time PCR Detection Kit was 97.14% for CT, 93.33% for MG, 95% for NG, 100% for TV, 95.95% for UU, 98.77% for UP and 99.74% for MH. The specificities were higher than 99% except for UP that was 97.6%.

Conclusion: This new assay fulfils the criteria of accuracy, sensitivity and specificity. In addition, compared with the "reference" commercial assay, the obtained results were faster and more reliable. A highlight feature of the evaluated product was that thanks to its lyophilized format, the manipulation was minimal, without intermediate mixtures, and was very useful for storage and transport at room temperature, instead of using refrigerated containers and occupying place in the freezers of the hospital.

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Time Dependent Apparent Increase in dd-cfDNA Percentage in Clinically Stable Patients between One and Five Years following Kidney Transplantation

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Background: Donor derived cell-free DNA (dd-cfDNA) has become a valuable tool for the surveillance of organ recipients. Several techniques are in use with different performance in larger clinical trials. Chronic antibody mediated rejection (ABMR) has been shown to be the major cause of allograft loss. Early detection of ABMR using dd-cfDNA might be valuable, since about 10-20% of rejections are subclinical. A possible change in reference(threshold) values over time for dd-cfDNA has not been rigorously evaluated but seems very critical for long term monitoring of transplants. Our hypothesis was that graft independent changes in host cfDNA might confound dd-cfDNA percentage determinations.

Methods: We performed a prospective study with N=431 patients, of which N=303 patients were followed after kidney transplantation(KTx). Plasma was collected (n=940) at nine defined time points from 12 to 60 months after engraftment in a cross-sectional cohort of clinically stable patients. In each sample the total cfDNA in copies/mL plasma(cp/mL), dd-cfDNA percentage and dd-cfDNA concentration(cp/mL) were determined using droplet digital PCR. Upper percentiles (80th, 85thand 90th) were evaluated by quantile regression to assess a possible change of putative threshold values used to distinguish kidney graft injuries (e.g. rejection) from stable healthy KTx.

Results: Upper percentiles (80th, 85thand 90th) of the total cfDNA showed a significant decline over time of -2013, -3613, and -5098 cp/mL per log month (P=0.01367, P=0.00013 and 0.01299), respectively. Consequently, the dd-cfDNA (%) 85thand 90thpercentiles increased significantly by 0.23%, 0.41%, and 0.71% per log month (P<0.05, P<0.01 and P<0.01), respectively. For the 85thpercentile in this apparently stable group an increase from 0.63%(12 months) to 1.27%(60 months) was observed. In contrast the absolute copy numbers of dd-cfDNA were stable during the observation period (changes of 6 to 16 per log month; p>0.25 for all upper percentiles). An increase in white blood cells(WBC) was also observed, together with a negative time based interclass correlation of median WBC with the median concentration of tacrolimus. Whether this is a pharmacologic effect of reduced tacrolimus concentrations requires further investigation. However, it seems conceivable, since even after 60 months the median total cfDNA was still 1.4-fold higher (p<0.001) than the median in a group of patients (n=364) with other medical conditions not receiving tacrolimus; and 1.6-fold (p<0.001) compared to healthy controls (n=135). Our earlier determined threshold value of 52cp/ml was around the 80thand 85thpercentile of this stable KTx group and remained nearly constant over time

Conclusion: For the long-term surveillance of KTx, a precise knowledge of cfDNA time dynamics is crucial, but most reference values were stablished early after KTx. Our data show that the use of dd-cfDNA percentage alone could lead to a substantial increase of false positive results in long term patients. This could result in unnecessary biopsies, causing patients harm and increased healthcare costs. The observed time-dependency of dd-cfDNA percentage values in KTx strongly suggests that absolute rather than percentage of dd-cfDNA concentrations be used to avoid false positive results.

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Novel Cross-Platform High-Risk (hr) Low Occurrence HPV NAAT Positive Samples for Use in Nucleic Acid Detection as Prospective Quality Control

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Background: HPV genotyping nucleic acid amplification testing (NAAT) is becoming a cornerstone triage method for stratifying the risk related to cancer development in the infected patients. Moreover, monitoring of the general population for the high-risk HPV (hrHPV)/low occurrence types is becoming routine to determine vaccination efficiency and to evaluate oncogenicity of HPV types. The monitoring of low occurrence hrHPV types is done using full genotyping NAAT diagnostic systems. However, there is a lack of adequate QC material for full genotype testing, which affects the transition from traditional HPV testing to full genotype HPV testing. Currently, the main categories of positive samples used by clinical laboratories are either plasmid-based or cell line-based preparations. Both types have the major disadvantage of not complying with FDA recommendations for QC material to monitor the entire workflow for NAAT: nucleic acid extraction, amplification, and detection. The composition of current controls (especially plasmids) limits their use on certain NAAT platforms. In addition, they only share some characteristics of a patient sample specimen. Most of these controls do not contain enough exogenous matrix material to make the detection of HPV challenging, and do not cover the full scope of patient sample testing options such as; DNA, RNA, and protein (antigen) detection methods. The main objective of our study was to illustrate a cross-platform compatibility of novel high risk HPV (hrHPV)/low occurrence materials and their potential use in a clinical quality management system as QC samples. The positive samples for hrHPV types 31, 33, 39, and HPV 67 (hr Negative Sample) contain all the components normally found in an infected patient specimen, such as: integrated and episomal viral DNA, RNA, and proteins, as well as host epithelial cells. These features make these materials fully compatible with multiple NAAT diagnostic systems.

Methods: The hrHPV panel performance was evaluated in the Original Equipment Manufacturer (OEM) labs and internally in Microbix by using either a full genotyping tests (EUROIMMUN AG, BD Lifesciences), or by a genotyping tests that report low occurrence hrHPV by pooling results (Roche Molecular, Cepheid).

Results: Based on the studies, HPV Positive Samples for hrHPV types 31, 33, 39, and HPV 67 hr Negative Sample (hrHPV panel) contain all the diagnostic targets normally found in an infected patient specimen (integrated and episomal presence of viral DNA as well as host epithelial cells).The reported values for all the studies were categorized relative to the normal range of reported values for each test in order to compare the sample performance between diagnostic platforms.

Conclusion: The HPV Positive Samples formulated in a widely accepted sample transport medium, showed excellent compatibility with several hrHPV genotyping platforms. Overall, the performance of the hrHPV/ low occurrence panel in multiple NAAT methods targeting either sequences in the E6/E7 or L1 regions demonstrates the successful development of cross-platform compatible samples for low occurrence hrHPV detection.

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Genetic Diagnosis of a Zellweger Syndrome. A Case Report

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Background: The Zelleweger Syndrome is a rare congenital disorder characterized by low production or absence of peroxisones, specially in the liver and kidneys. This syndrome is caused by mutations in one of the 13 PEX genes, these genes encode peroxin proteins that are involved in different peroxisomal fuctions. Peroxisomes play an important role in numerous metabolic processes, but primarily the metabolism of fatty acids. Because of the defect in peroxisome formation, multiple metabolic pathways are impaired resulting in metabolic abnormalities like accumulation of very long chain fatty acids and other lipids, as in the deficiency of certain macromolecules metabolized by peroxisomes, such as plasmalogens.This syndrome is suspected in the physical examination of the patient and the plasma elevation of very long chain fatty acids. These patients present with hypotonia and seizures shortly after birth, neuronal migration deficiencies, dysmorphic features, liver disease, retinal degeneration, sensorineural deafness, polycystic kidneys, and high blood levels of minerals such as iron and aluminum. Regardless of the interventions, the prognosis is unfavorable and most patients die in the first year of life due to respiratory failure associated with an infection or intractable epilepsy.

Methods: The patient was a new born that in the prenatal ultrasound presented choledochal cyst, interhemispheric cyst and small interventricular cysts. At birth he was atmitted at Neonatology due to mild respiratory distress and hypoactivity. The patient also had severe axial hypotonia, bilateral cryptorchidism, bilateral talipes equinovarus feet and syndactyly between second and third finger of the left foot. Facial features that were suspicious presented macrocephaly, broad nasal root, descending palpebral fissures, ears of low implantation, corneal opacification and cervical asymmetries. A biochemical analysis highlighted the increase in long-chain fatty acids and ferritin, in addition to the decrease in cholesterol levels.Given the diagnostic suspicion of Zellweger Syndrome, a molecular study was initiated by massive sequencing for the analysis of small deletions / insertions and point mutations in the coding region and splicing sites of the PEX1, PEX6, PEX26 and PEX10 genes.

Results: By direct analysis of the above genes, a transition in the PEX26 gene of a thymine through a cytosine at position 134 was detected in the patient. This produces at the protein the change of the amino acid leucine from position 45 for a proline, being a pathogenic change associated with a disorder in the biogenesis of peroxisomes. The results concluded that the patient was apparently a homozygous carrier of the pathogenic change c.134T>C (p. Leu45Pro) in the analyzed sequence of the PEX26 gene, being compatible with his clinical diagnosis of Zellweger syndrome.

Conclusion: The clinical presentation does not always lead to a quick diagnosis in the newborn, and may be confused with other diseases such as Infantile Refsum disease or neonatal adrenoleukodystrophy. Due to this, the genetic study is of vital importance in these cases.The analysis of the c.134T>C change in the PEX26 gene in the patient's parents is recommended in order to determine their carrier status and confirm the homozygosity of said change in the patient.

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Case Report: Maternal Balanced Translocation Generating an Offspring with an Apparent Balanced Translocation and Partial Trisomy 21 due to a Cryptic Duplication on Chromosome 21

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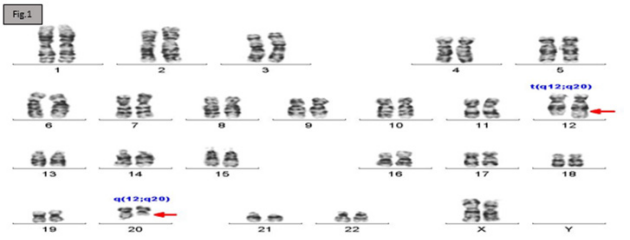
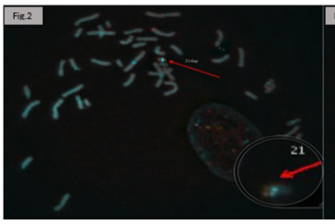
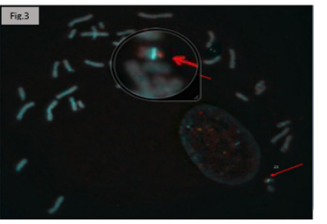
Background: We describe a case of a 31 year old pregnant woman (G2, P1, A0, L1) who underwent an invasive antenatal screening with a chorionic villus biopsy at 12th WG due to a high trisomy 21 risk (1/24), after a first trimester combined screening test. **Objective:** To describe a mismatch between an unusual G-banding karyotype and an altered array-CGH. **Methods and results:** Chorionic villi sample were implanted in T-25 tissue culture flasks with an in-vitro culture medium. After the cells confluency, they were harvested and fixed for conventional cytogenetics. The karyotype showed an apparent balanced reciprocal translocation of chromosomes 12 and 20, approximate breakpoints q24;q12. t(12;20)(q24.3;q12). (Fig.1). Chorionic Villi Array-CGH (60K) showed a duplication of the region 21q22.12q22.13 and 21q22.3 terminal area. Maternal Karyotype from peripheral blood showed the same balanced reciprocal translocation. FISH with chromosome 21 critical region (21q22 probe) was normal. Father Karyotype was normal. At the 16th WG, the prenatal ultrasound was normal and an amniotic fluid sample was obtained in order to rule out placental mosaicism. A second Array-CGH was performed in amniocytes, confirming the results from the villus biopsy. Fluorescence in situ hybridization with 21q22 probe (LSIAML 21q22 spectrum aqua DNA probe), in cultured amniocytes showed a telomere cryptic duplication of 21qter Ish.der(21)(subtel21q++). (Fig.2 and 3). **Conclusions:** The complex rearrangement with the result of a partial trisomy 21 (dup21qter) is considered extremely rare and could have originated by an anomalous segregation of the translocated chromosomes at meiosis. The duplication overlaps the Down syndrome critical region, including DYRK1A gene. The expected phenotype could be a milder form of Down syndrome. This case demonstrates the need to use the entire available conventional cytogenetic test in order to achieve a correct diagnosis.







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Novel Mycoplasma genitalium (Mgen) and AMR Mgen A2059G Materials for use in a Cross-Platform NAAT Quality Control

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Background: *Mycoplasma genitalium* (Mgen) nucleic acid amplification testing (NAAT) is emerging as a cornerstone triage method for sexually transmitted infection evaluation in the infected population. Furthermore, the introduction of antimicrobial resistance (AMR) Mgen evaluation NAAT step is needed and becoming mandatory in certain geographies. However, the placement of currently available NAAT tests on the market is affected by the lack of proper quality control (QC) material, and may impact timely testing and patient diagnosis. The main categories of QC samples currently used by clinical labs are either plasmids or patient samples. These types of controls are not fully compliant with the FDA /CE recommendations for QC material to monitor the entire test workflow (nucleic acid extraction, amplification, and detection) and to be non-infectious and repeatable.

Methods: The performance of novel Mgen and AMR Mgen controls containing all the components found in the infected patient specimen (human cells, bacterial DNA and RNA) was evaluated in the Original Equipment Manufacturer (OEM) laboratories and internally by various NAAT detection methodologies.

Results: Data from the participating OEM labs and form the internal testing (Diagenode) shows cross-platform compatibility of the Mgen and AMR Mgen material used in the study (Table 1).

Conclusion: Novel Mgen and AMR Mgen materials formulated in a widely accepted sample transport medium showed excellent compatibility with several OEM platforms utilizing TMA, qPCR and TOCE-DPO detection methods. The successful detection of various DNA and RNA targets is demonstrating the achievement of constructing cross-platform compatible Mgen and AMR Mgen samples for use as prospective quality controls.

OEM	Test	Platform	Target	Mgen	
				G37	A2059G
Hologic	Aptima Mycoplasma genitalium	TMA	RNA	+++	NA*
Seegene	Allplex™ CT/NG/MG/TV Allplex™ MG&AZIR	TOCE-DPO	DNA	+++	+++
Roche	cobas® TV/MG	qPCR		+++	NA*
Elitech	STI PLUS ELITe MGB®			+++	NA*
SpeeDx	ResistancePlus® M G			+++	+++
Diagenode	Diagenode S-DIAMGTV / Diagenode S-DiaMGRes			+++	+++

Table 1. Results from testing Mgen and AMR Mgen on various platforms. (NA* - test is not intended to detect the AMR Mgen mutants)

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Comparative Study of Restriction Fragment Length Polymorphism PCR and Real Time PCR Molecular Methods for Identification of rs4988235 Polymorphism

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Background: Lactose, the main carbohydrate in mammalian milk, is hydrolyzed by the enzyme lactase in the small intestine. Lactase activity is reduced after weaning in most people, and is therefore expressed in small amounts during adulthood, a condition called primary hypolactasia or non-persistent lactase. Several studies have shown that the rs4988235 (LCT -13910 C/T) polymorphism of the MCM6 gene is strongly correlated to the persistence of lactase enzyme in adulthood. This gene is located about 14Kb upstream of the lactase (LCT) gene and is responsible for transcriptional activation of the LCT promoter. Currently, molecular assays such as Restriction Fragment Length Polymorphism PCR (RFLP PCR) and Real Time PCR (qPCR) are some of the most used for the determination of genotypes; however, there are several differences between these methodologies when comparing sensitivity, quality of reaction and hands-on and processing time, for example. **Objective:** To compare two molecular methodologies for determination of genotypes of rs4988235 polymorphism.

Methods: Genomic DNA samples from 156 individuals previously genotyped by Restriction Fragment Length Polymorphism PCR were used to validation. DNA isolation was performed with the automated platform Qiasymphony SP (Qiagen, Hilden, Germany) according to manufacturer instructions. Genotyping for rs4988235 was performed using TaqMan® SNP Genotyping Assays (Life Technologies, Foster City, CA) at 7500 Fast Real-Time PCR System (Life Technologies). The results were analyzed using TaqMan® Genotyper Software version 1.0.1. **Results:** Carriers of rs4988235 CC genotype predominated (91/156, 58.3%), heterozygotes were 46/156 (29.5%) and the remaining homozygotes for allele T were 19/156 (12.2%). The results showed agreement and reproducibility of 100% for both methodologies. Several differences in workflow were observed between the two LCT assays methodologies. The qPCR assay showed good concordance with the RFLP PCR assay; however, in the qPCR assay, the reaction quality was higher, and the execution time was smaller (four hours and thirty minutes for qPCR and thirteen hours for RFLP PCR). **Conclusion:** The qPCR assay is a sensitive and reliable LCT genotyping assay. The workflow of this molecular assay results in higher throughput, improved efficiency, and a decreased time to release results. Compared to other genotyping methods, such as PCR-RFLP, this method is easy to perform and useful in routines with a high number of samples to be processed. The results found equate with the frequency of SNPs established by previous literature for the global population.

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Evaluation of a Pharmacogenomics Panel on the TaqMan OpenArray Genotyping Platform

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Background: Since 2011, the Clinical Pharmacogenetics Implementation Consortium has published genotype-based guidelines for genetic variants which have sufficient evidence available for the use of pharmacogenomics (PGx) information in clinical settings. We validated a custom-designed OpenArray™ PGx panel (OA-PGx panel), which uses the TaqMan allelic discrimination assay format, for genotyping of 480 single-nucleotide polymorphisms (SNPs). This panel targets variants involved in drug efficacy (*e.g.* absorption, distribution, metabolism and excretion) and safety. It is currently used in clinical trials employing PGx for decision-making.

Methods: 43 Coriell Institute cell line DNA samples (CCL samples) and DNA isolated from 33 whole blood samples were used in accuracy, precision and sensitivity studies for 480 SNPs on the OA-PGx panel. For accuracy evaluation using CCL samples, the genotyping calls from the OA-PGx panel were compared to their respective genotypes in the 1000 Genomes Project. For accuracy determination of whole blood sample DNA, the OA-PGx panel results were compared to results from Sequenom MassARRAY® (Oregon Health & Science University) or next generation sequencing (Molecular Diagnostic Laboratory, University of Chicago). For precision evaluation, 23 CCL samples were analyzed three times. For sensitivity evaluation, 6 CCL samples and 5 whole blood samples were analyzed using 10 ng/µL and 50 ng/µL DNA.

Results: 439 SNPs passed accuracy, precision and sensitivity tests. The passing rates in accuracy, precision and sensitivity tests were 93.4% (440/471), 98.8% (474/480) and 99.8% (479/480), respectively. 41 SNPs were not validated as 32 SNPs failed accuracy and/or precision, and for 9 SNPs there was no reference genotype available. 1 SNP failed sensitivity due to an incorrect call made by the 10 ng/µl DNA run for one of the samples; however, this SNP is still considered validated since 50 ng/µL DNA is used on the OA-PGx panel. There were 5 SNPs that passed accuracy, precision and sensitivity tests, but showed various performance issues (*e.g.* low amplification).

Conclusions: We performed the analytical validation of a custom-designed OA-PGx panel with 480 SNPs. It is one of the largest panels implemented to date. This study demonstrated that the OA-PGx panel provides reliable SNP genotyping results for 434 SNPs which are implicated in precision medicine.

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Study of Circulating miR-519d and miR-26b as Potential Therapy-Induced Autophagy Markers of Chemoresponse to Conventional Doxorubicin-Based Superselective Transarterial Chemoembolization in Egyptian Patients with Hepatocellular Carcinoma on Top of Hepatitis C Virus Infection

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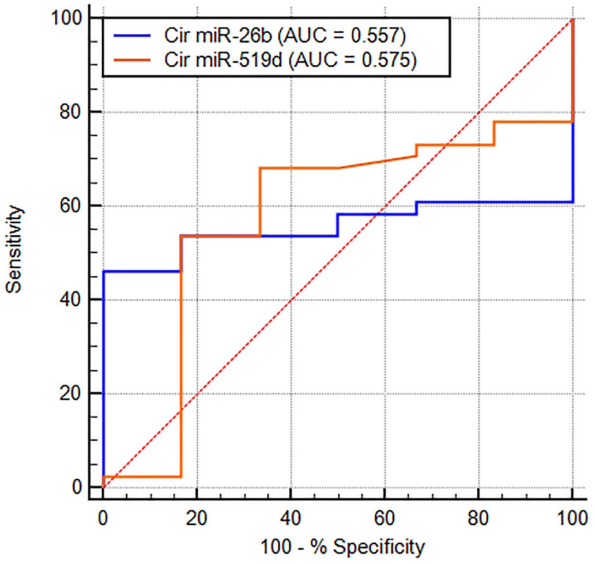
Background: Doxorubicin-based superselective transarterial chemoembolization (ssTACE) has emerged as a bridging therapy for early stages of hepatocellular carcinoma (HCC) awaiting liver transplantation. Clinical resistance to doxorubicin is a major problem that involves multiple molecular mechanisms, including therapy-induced autophagy. Recently, several miRNAs as miR-519d and miR-26b have been found to be involved in autophagy modulation. This study aims at assessing the diagnostic accuracy of circulating miR-519d and -26b as potential therapy-induced autophagy markers of response to doxorubicin-based ssTACE in Egyptian HCC patients on top of chronic hepatitis-C viral (HCV) infection. **Methods:** Forty-seven HCV infected HCC patients were referred for doxorubicin-based ssTACE, and after three months follow up period resulted in forty-one responders and six non-responders based on modified response evaluation criteria in solid tumors (mRECIST).Twenty matched individuals were included as a reference group for miRNA expression calculation. Pre and post ssTACE tumor volume and viability were assessed using a computerized tomography scan. Circulating pre ssTACE miR-26b and -519d expressions were determined using real-time quantitative polymerase chain reaction. **Results:** Circulating miR-26b but not miR-519d was significantly upregulated in HCC patients (median = 4.56) compared to the reference group (median = 1.81), p=0.017. However, there was no statistically significant difference between responders and non-responders in terms of miR-519d or miR-26b expression level (p=0.5, p=0.6), respectively. Post ssTACE tumor volume was significantly lower than pre ssTACE in responders, p <0.001. None of the miRNAs (miR-519d,-26b) was found as a significant discriminator to chemoresponse by Receiver operating characteristics (ROC) curve analysis with an area under the curve (AUC) of (0.575, 0.557) respectively and p-value of (0.556, 0.463) respectively. **Conclusion:** Although miR-26b was significantly upregulated in HCC cases compared to the reference group, However, there is no significant association between the expression level of both miR-519d or -26b and response to Doxorobicin-based ssTACE. A large scale study involving other chemosensitive/chemoresistant miRNAs is highly recommended.



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Development and Validation of a Multiplexing Digital PCR Spinal Muscular Atrophy (SMA) Screening Assay on a Novel Integrated Digital PCR Instrument

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Introduction: Spinal muscular atrophy (SMA), a genetic neuromuscular disorder and historically a leading genetic cause of infant mortality, is largely caused by the loss of the *survival motor neuron 1* (*SMN1*) gene. The duplicated *SMN* gene, *survival motor neuron* 2 (*SMN2*), has been found to produce partial function and can partially compensate for *SMN1* deletion and reduce SMA disease severity. Accurate quantification of *SMN1* and *SMN2* copy numbers provides critical diagnostic and prognostic values for the disease. SMA has become an actionable disease with the recent advancement of treatments. Newborn screening of SMA is increasingly practiced in many states and other countries. Current screening methods are complex and time consuming. Here we present a simple high-level multiplexing digital PCR solution on a novel digital PCR (dPCR) platform to meet the growing demand for SMA newborn screening and treatment decisions.

Methods: The hydrolysis probe-based multiplexing digital PCR assay quantifies *SMN1* (FAM), *SMN2* (HEX) and the total *SMN* (*SMN1* + *SMN2*) (TYE665) targeting the intron 1 region, while using *RPPH1* gene (TAMRA) as a 2-Cp internal reference control. We first evaluated the 4-plex assay on characterized control DNA samples and then we validated the assay with 15 blinded clinical samples. We also simplified the experiment by eliminating restriction enzyme digestion usually recommended for copy number detections. All experiments were conducted on a novel digital PCR instrument with 5 optical channels and a walk-away workflow identical to real-time PCR. The digital PCR integrated partitioning of PCR mixture, thermocycling and data acquisition into a single bench top instrument. The copy numbers were determined by an analysis software using Poisson statistics on the total, negative and positive partitions.

Results: Our multiplexing digital SMA screening assay showed high specificity and sensitivity using the control samples with *SMN1*/*SMN2* copy numbers at 0:3 (NA23255), 3:0 (NA17117) and 1:1 (NA03815). The copy numbers of the fifteen blinded clinical samples were correctly identified with 100% concordance to a previously published study including a 5-Cp *SMN2* copy number sample. Additionally, the total *SMN* from 14 blinded clinical samples agree with the sum of the copy numbers from *SMN1* and *SMN2* assays, indicating there is no additional deletion downstream of exon 7. One clinical sample showed the total copy number (4 copies) is more than the copy numbers from *SMN1* (zero copies) and *SMN2* (2 copies) due to a microdeletion. This sample has two *SMN1* alleles that are lacking exons 7 and 8 (SMN[DELTA]78) resulting from a 6.3 kb microdeletion of *SMN1* instead of a full deletion of *SMN1*. These microdeletions were verified by regular PCR. Additionally, the multiplexing dPCR SMA copy number screening assay can be accomplished within 90 minutes on the integrated platform, potentially allowing the assay to be translated into a clinic setting.

Conclusion: We developed a truly rapid multiplexing SMA screening assay with a simple workflow on a fully integrated digital PCR instrument. This multiplexing digital PCR assay provides a highly accurate, sensitive and powerful solution for SMA newborn screening and therapeutic treatment decisions.

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Impact of the Classification of Genetic Variants in the Clinical Laboratory Routine: Comparison between Different Tools Using 2015 ACMG Guidelines

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Background: In recent years, the expansion of new generation sequencing (NGS) has allowed a substantial increase in the number of genetic tests in clinical laboratories. This change was accompanied by new challenges in the classification of genetic variants, which has become one of the most complex and laborious tasks in the laboratory routine. Studies have revealed a high disagreement between classifications, reaching 21% intra-laboratory and 66% inter-laboratory. In 2015, the American College of Medical Genetics and Genomics (ACMG) published guidelines in order to systematize and reduce subjectivities in the classification process. **Objective:** Analyze the classification concordance between curatorships of molecular genetics specialists of the Hermes Pardini (HP) group with other classification tools: ClinVar; another International clinical laboratory (Z); two conventional commercial software (X and Y) and two machine learning-based software (Franklin and PrimateAI).

Methods: The five main ACMG classes, Pathogenic (PAT), Likely Pathogenic (LP), Variant of Unknown Significance (VUS), Likely Benign (LB) and Benign (BEN) were grouped in three categories LP/PAT, VUS and LB/BEN. All HP genetic variants were classified according to the ACMG guideline independently by two specialists. Any inconsistencies were further reviewed. In a first experiment, 70 (14 LP/PAT, 47 VUS, 9 LB/BEN) HP variants were compared with Clinvar, X, Y and Z. Secondly, 329 (24 LP/PAT, 91 VUS, 214 LB/BEN) and 333 (24 LP/PAT, 94 VUS, 215 LB/BEN) HP variants were respectively compared with Franklin and PrimateAI. All software were used with default parameters. ClinVar results were retrieved online. **Results:** Experiment 1: Our analysis showed that only 52% of the ratings were consistent among all tools. Furthermore, for LP/PAT variants, our classification was 100% consistent with Z, 81% with Y software, 76% with ClinVar and 73% with X software. For VUS, we had 59% agreement with software X, 52% with software Y, 46% with ClinVar and 41% with Z. For LB/BEN variants, 37% was in agreement with the X software and only 20% with Z. For these variants, there were no agreement with the classification of ClinVar and the software Y. Experiment 2: For LP/PAT, VUS and LB/BEN variants, our classification was respectively consistent with Franklin and PrimateAI in 92%, 13%; 77%, 29% and 83%, 91%. **Conclusion:** Our result showed that the classification could not be defined in an automated way by a single classification tool. Although they all have their limitations, they prove to be useful, helping and even streamlining this process, which must be done by a multidisciplinary group of specialists, with expertise, knowledge for discussion, refined evaluation and curation of the genetic variants identified in laboratory practice. Machine learning-based software could be of great value in the field.

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Improving Clinical Diagnosis and Therapeutic Orientation of Metabolic Findings with Molecular Analysis of Patients with Inborn Errors of Metabolism

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Background: The clinical manifestations of Inborn Errors of Metabolism (IEM) in the neonatal period can mimic other more frequent pathologies leading to possible confusions, delays in diagnosis and the initiation of therapies that may hinder the specific metabolic investigation. **Objective:** Describe the evolution of a patient in a Neonatal Intensive Care Unit of a university hospital in the city of São Paulo, with a severe and complex condition, which despite altered metabolic examination, its diag-

nosis and therapeutics could only be safely confirmed after the molecular investigation of the newborn and their parents. **Case Report:** The patient was a term female newborn, the first of a young non-consanguineous couple. During the gestation, the mother presented, active Lupus, pulmonary hypertension and used several immunosupressors. On second day of life, the newborn presented a severe gastrointestinal bleeding with hypovolemic shock, hepatomegaly, increased prothrombin time and aminotransferases. To control the clinical situation, she received blood transfusions. On 4th day, *Cytomegalovirus* was detected in the urine, and she started therapy with ganciclovir. After 2 days, she maintained the clinical condition, had edema on the legs and ascites. At that moment, the team of clinical geneticists performed an evaluation. On 8th day, the neonatal screening test (collected before blood transfusions) revealed an increase in galactose and a decrease in GALT activity. Immediately, lactose-free diet was introduced and molecular analysis was performed. **Methods:** Genomic DNA was extracted from buccal swab. *GALT* gene sequencing was performed on the Illumina NextSeq 500 platform, according to the manufacturer's instructions. Variants pathogenicity were assessed according to the ACMG/AMP recommendations. Proband parents' were also investigated for *de novo* mutation analysis. **Results:** Two heterozygous mutations were identified on *GALT* gene: a pathogenic mutation in exon five (rs111033690; NM_000155.4:c.404C>T; p.Ser135Leu) and a likely pathogenic in exon nine (NM_000155.4:c.895G>A; p.Gly299Ser). Parents' research revealed trans variants, identifying the molecular profile of the patient as a compound heterozygote, corroborating the clinical diagnosis and the therapy maintenance. The evolution was only favorable with disappearance of edema within 30 days of life. **Conclusion:** In complex and rare diseases, the rapid diagnostic and parallel research for distinction from common causes that can mimic it are of the utmost importance. It is common for the affected patients to receive blood transfusions, preventing metabolic investigations. Therefore, molecular research presents as a resolutive tool.

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Diagnostic Performance and Validation of a New Molecular Method for the Identification and Differentiation of Tuberculous and Non-Tuberculous Mycobacteria in Respiratory Samples

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Background: Human tuberculosis (TB), caused by infection of *Mycobacterium tuberculosis*, continues to be a major cause of morbidity and mortality worldwide, causing around 1.5 million deaths and 10 million cases each year. The early diagnosis of this disease is essential for its control. The gold standard in laboratory diagnosis of TB is direct microscopic examination and culture of the sample. However, culture of *M. tuberculosis* can take up to 8 weeks and in 10-20% of cases is unsuccessful. Molecular methods can ease a fast and adequate establishment of diagnosis. Therefore, the objective of this study is to evaluate the accuracy of a recently developed qPCR detection kit.

Methods: An External Evaluation has been conducted in the Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Barcelona (Spain) to assess the performance of "VIASURE M. tuberculosis complex + non-tuberculous mycobacteria Real Time PCR Detection Kit" (Certest Biotec, Spain). Briefly, DNA from 130 decontaminated sputum specimens (49 respiratory bacterial infection specimens, 30 respiratory NTM infection specimens, and 51 TB specimens) were analysed. The results were compared with culture identification results and the qPCR commercial kit Anyplex MTB/NTM (Seegene, Korea). Viasure PCR and Anyplex MTB/NTM values of sensitivity and specificity (95% confidence interval), agreement values and kappa coefficients were calculated considering as reference method culture identification using SPSS 15.0 (SPSS Inc, USA).

Results: Viasure PCR detected correctly 33/49 non-mycobacterial specimens with a resulting specificity of 67.3% when compared to culture identification. Viasure detected correctly 23/30 (76.7%) NTM and 50/51 (98.0%) of the MTBC samples. Agreement between Viasure and culture identification was 81.5% (106/130), with a high kappa (0.717). The agreement between Viasure and Anyplex MTB/NTM was 77.7% (101/130), with a high kappa (0.639). In specimens from patients with a respiratory bacterial infection and negative for NTM and MTBC, 16 false positive results (FP) were obtained with Viasure and 13 with Anyplex MTB/NTM, 8 of which were detected as MTBC with both tests. In addition, in specimens that were culture positive for NTM, 4 FP (detection of MTBC or *M. tuberculosis*) were obtained with Viasure and 7 with Anyplex, one being detected as MTBC with both tests. In most of the false positive results for both molecular assays, residual amplifications with Ct<35 were obtained. PCR contamination is unlikely since negative controls were valid, however, contamination in initial steps of sputum processing cannot be ruled out. Comparing both molecular assays, regarding the detection and identification of NTM, Viasure was more sensitive than Anyplex MTB/NTM (76.7% and 60.0%, respectively), but less specific (91.0% and 100%, respectively). Regarding the detection and identification of MTBC, Viasure was slightly less sensitive than Anyplex MTB/NTM (98.0% and 100%, respectively), but more specific (84.8% and 74.7%, respectively).

Conclusion: The evaluated method provided a good correlation with the routine analysis and other commercial assay, providing a time advantage compared to routine analysis. A highlight feature specific to the evaluated product was that thanks to its lyophilized format, the manipulation was minimal and was very useful for storage and transport at room temperature.

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Laboratory Findings in a Large Cohort of Patients with Homocysteine Remethylation Disorders and Cystathionine-Beta-Synthase Deficiency

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Background: Several treatment modalities are utilized to reduce homocysteine toxicity and normalize methionine levels in inherited disorders of sulfur amino acid metabolism, including the use of hydroxycobalamin/betaine in remethylation disorders and low-methionine diet in combination with betaine in cystathionine-beta-synthase (CBS) deficiency. Total homocysteine (tHcys), methionine and, in patients with combined methylmalonic acidemia/homocystinuria, methylmalonic acid (MMA) are routinely measured in plasma to monitor patient's response to treatment, but only few studies correlated biochemical data with different therapies. Here we report the long-term follow-up of laboratory testing data in a large cohort of patients with different disorders of sulfur amino acid metabolism. **Methods:** This study retrospectively analyzed results for amino acids, tHcys and MMA in 812 plasma samples from 56 patients with remethylation disorders and 67 patients with CBS deficiency. Plasma amino acids were analyzed by liquid chromatography tandem mass spectrometry using aTraq™ reagents (Sciex, Framingham, MA, USA). Plasma tHcy was quantified using the Diazyme enzymatic assay (Roche cobas c502). MMA was measured by high performance liquid chromatography mass spectrometry. Data was analyzed using Prism v.8.3.0 software (La Jolla, CA). The study was approved by the Institutional Review Board of the University of Utah. **Results:** In most patients, treatment decreased, but did not normalize tHcys, with the highest levels seen in CBS and MTHFR deficiencies (116±79µmol/L v.s. 102±56µmol/L; normal range ≤10µmol/L). In CBS deficiency, tHcys correlated positively with methionine (r_s=0.51, p<0.0001) and inversely with cystine (r_s=-0.57, p<0.0001), which is consistent with a metabolic block in the trans-sulfuration pathway. Normal methionine was observed only in 16% of samples from CBS deficiency patients compared to 81% of samples from patients with remethylation disorders. In the latter group, the inverse correlation of methionine with tHcys (r_s=-0.57, p<0.0001) suggested increased activation of tHcys remethylation by hydroxycobalamin and/or betaine. Positive correlation between MMA levels and tHcys in patients with combined methylmalonic acidemia/homocystinuria (r_s=0.57, p<0.0001) demonstrated that hydroxycobalamin was also effective in lowering MMA levels. Betaine increased the levels of another amino acid, sarcosine: 2±3µmol/L vs 19±7µmol/L in MTFHR deficiency; 2±3µmol/L vs 14±5µmol/L in CBLC deficiency; 7±6µmol/L vs 19±15µmol/L in CBS deficiency. Furthermore, in patients with remethylation disorders sarcosine >5µmol/L (normal range <5µmol/L) showed 97% sensitivity and 95% specificity as marker of betaine supplementation, superior to tHcys and methionine, which are traditionally used for evaluation of treatment efficacy. The area under the receiver-operating curve (AUC) for sarcosine was 0.98 (p<0.0001) compared to 0.83 (p<0.0001) and 0.51 (p=0.8254) for tHcys and methionine, respectively. Sarcosine also provided a significantly better discrimination between CBS deficient patients treated or not treated with betaine (p<0.0001; AUC=0.86) compare to methionine (p=0.7672; AUC=0.52) or tHcys (p=0.1273; AUC=0.60). **Conclusion:** In conclusion, biochemical testing is helpful in monitoring the effect of different therapies in patients with inherited disorders of sulfur amino acid metabolism. Sarcosine can be used for evaluation of compliance to betaine treatment.

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Customization of Next Generation Sequencing Panel Optimized for Analysis of Cancer-Related Genes

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Background: The sequencing of the KRAS, BRAF, NRAS and EGFR genes allows the targeting for the treatment of some types of cancer, especially lung and colorectal cancer, which are among the five most common types of cancer in Brazil. Due to advances in the identification of mutations and the emergence of new drugs for treatment, there is a constant need to update these genetic panels. **Objective:** Customize a new panel to improve the regions of clinical importance commonly studied. **Methods:** For comparison between different sets of primer pools by Next Generation Sequencing (NGS), we selected 31 samples with known results (eight randomly and 23 with specific variants of interest) to be analyzed by NGS and pyrosequencing. The criteria used to compare the different pools by NGS were the base coverage (depth) and the reproducibility of the genotypes. For the pyrosequencing comparison, we analyzed the reproducibility of the genotypes. **Results:** The agreement between the genotypes of the samples analyzed by NGS in the two panels (current and new primers pools) was 100% and the overall coverage of the panel increased, on average, 3%. The coverage of the regions of interest showed no significant differences ($p < 0.01$). The new panel included new regions of interest and excluded regions without clinical relevance, but did not cover seven codons from EGFR exon 21, 1 from exon 20 and 2 from exon 19; however, they are still under study. When compared to pyrosequencing, the genotyping of the samples was approximately 74% consistent and only 6% disagreed. About 14% of the samples were not conclusive in NGS, while 6%, in pyrosequencing, probably due to the inherent degradation of the material used. In 3% of cases, due to the complexity of the alteration, only genotyping by NGS was possible. **Conclusions:** The panel update met the proposed objectives with good quality parameters, including codon 117 of the NRAS gene and codon 797 of the EGFR gene, which are clinically relevant regions. New studies will be proposed to include codons not covered in the new panel, meeting the demand of physicians.

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Rapid and Sensitive Detection of Orthopoxviruses by Real Time Polymerase Chain Reaction Test using the Portable Thermocycler T-COR 8™

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Background: Orthopoxviruses (OPV) are large enveloped DNA viruses belonging to family *Poxviridae*, genus orthopoxvirus (OPV). OPV genus includes closely related viruses viz variola virus (VARV), and other zoonotic viruses viz. Vaccinia virus (VACV), Monkeypox (MPXV), cowpox (CPXV), and camel pox virus. These viruses may cause human febrile disease with a rash, which may range from being a benign lesion to a severe fatal systemic infection such as smallpox. Despite the eradication of smallpox, circulating zoonotic viruses remain a threat to human health due to the increasing number of unvaccinated people. Thus to meet the need for sensitive, rapid, and near-patient detection of OPV, we have evaluated our dried-down real-time polymerase chain reaction (RT-PCR) test for portable thermocycler T-COR 8™. Methods: This assay includes dried down reagents to amplify OPV DNA and synthetic target as an internal control (IC) to monitor the test performance. We have developed a simple sample processing protocol and a self-metering cartridge with dried down reagents for field use. Analytical characteristics of the assay were determined using three different preparations of Modified Vaccinia Ankara (MVA) strain obtained from BEI resources. We tested gamma-irradiated VACV, MPXV, and two different CPXV strains, namely CPXV (Norway) and CPXV (Germany), to determine the inclusivity characteristics. The performance of a simplified sample collection device was also evaluated in this study for direct testing of samples without any DNA extraction. Results: We observed that the assay was able to detect the three different preparations without any nucleic acid extractions in the range of 10-390 pfu/mL. Evaluation of an enclosed sample collection swab was performed using different spike volumes of the target OPV preparations. We were able to detect all four types of OPVs in the sample. The recovery of amplifiable DNA was higher when spiked at 5 µL per swab vs. 1 µL / swab when dilution factors remain the same as 1:400 in the sample collection device. Conclusion: The T-COR 8 device can be successfully used in the field for a simple, extraction free, rapid, and sensitive detection of OPV DNA in samples. We plan to evaluate this system further using different clinical matrices.

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Interfacing Clinical Impressions, Clinical Chemistry and Molecular Diagnostics towards the Goal of Developing Standardized Diagnostic Algorithms for Incorporation of Whole Exome Sequencing (WES) in Clinical Practice

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Background: The American College of Medical Genetics (ACMG) recommends considering the whole exome sequencing/whole genome sequencing (WES/WGS) for assessment of phenotypically affected individuals when i) genetic etiology is implicated through phenotype or family history, but no targeted genetic is testing available; ii) a defined genetic disorder is present, but is associated with a high degree of genetic heterogeneity; and iii) targeted/specific genetic tests have not resulted in a diagnosis despite a probable genetic disorder. As it becomes more technologically and financially accessible, clinical teams are more readily incorporating WES in the diagnostic workup of their patients. Recent studies estimate the diagnostic utility of WES to be 36% in a pediatric population. However, the vast implementation of NGS methods has resulted in a rapidly progressing field, yielding continuous improvement in genotype-phenotype correlations that inform the clinical relevance of WES results. The limitations and changing landscape of information surrounding NGS methodology in clinical practice emphasizes the importance of interpreting WES in the context of additional relevant diagnostic tools. Here we describe a multidisciplinary approach to evaluate the diagnostic performance of WES in the pediatric population of a large academic medical center, towards the goal of identifying a standardized laboratory testing algorithm for WES, which incorporates clinical symptoms and phenotype, patient and family history, and relevant diagnostic testing. **Objective:** To use a multidisciplinary approach to identify a standardized laboratory testing algorithm for WES in a pediatric population. **Methods**: Pediatric WES test information was acquired by a query of the University of the Kentucky Medical Center (UK) billing data from October 2018 to December 2019. All oncology patients were excluded. Clinical information including phenotypic presentation, family history, biochemical and molecular analyses were retrieved from the patient electronic medical records. Data was evaluated by an interdisciplinary team including specialists in clinical chemistry, pediatric genetics, genetic counselling and molecular diagnostics. WES data was deemed diagnostically relevant based upon variant definitions outlined in the 2015 ACMG/Association for Molecular Pathology guidelines and if the results were clinically actionable. **Results:** An initial analysis of N=60 pediatric patients showed a positivity rate of 30% (including both pathogenic and likely pathogenic variants), 45% of the results were considered variants of undetermined significance (VUS), and the overall the negative rate, i.e. no reportable variants, was 18.3%. For a subset of WES orders, an interdisciplinary team reviewed patient data at the time of order and made formal recommendations regarding WES testing to the genetic counseling team, yielding an improved positivity rate of 67% for pathogenic variant detection. **Conclusions:** While all genetic testing was under a formal review process, the practice was directed towards more focused genetic analysis. Retrospective review of pediatric WES data revealed an overall positivity rate of 30%. The improved diagnostic yield of WES when interdisciplinary team review process was implemented favors the use of standardized testing algorithms that incorporate clinical phenotype, symptoms, relevant biochemical and molecular diagnostic information, and the perspective of genetic and clinical laboratory experts to improve the diagnostic yield of WES testing in clinical practice.

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Development of Orthogonal and Customized Molecular Methods for Genetic Variants Detection in Family Members of Individuals with Diagnosis of Pathogenic Mutations

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Introduction The advent of massively parallel sequencing (MPS) has popularized human genome analyzes and an increasing number of investigations and diagnostic confirmations of inheritable diseases have been carried out. The discovery of a pathogenic or probably pathogenic variant in an index case qualifies their closest relatives to the investigation of the mutation in question in order to estimate their risk for the clinical condition and the necessity of genetic counseling. This situation implies the development of a custom orthogonal method for the family so that the variant can be tested in a large number of individuals in a cost-effective way. In this study, we report the investigation of pathogenic variants through allele-specific qPCR customized for two Brazilian families whose index cases were diagnosed with pathogenic

variants by SMP. **Method** The index case of the first family had a diagnostic hypothesis of hereditary amyloidosis (MIM 105210) and was identified with the variant c.424G>A (p.Val142Ile) in heterozygosity in the TTR gene. These variants are classified as pathogenic in Clinvar (VCV000013426.10). Heterozygous mutations in the TTR gene are associated with hereditary transthyretin-related amyloidosis characterizing an autosomal dominant inheritance. The same mutation was identified in the individual's mother. Eight uncle/aunts, 2 brothers, 5 nephews, 18 first cousins and 11-second cousins totaling 31 subjects agreed to perform the customized assay. The index case of the second family was diagnosed with multiple endocrine neoplasia IIA (MIM 171400) and was identified with the variant c.2753T> C p. (Met918Thr) in heterozygosity in the RET gene. This variant is classified as pathogenic in Clinvar (VCV000549822.1). Heterozygous mutations in RET gene are associated with multiple endocrine neoplasia IIA characterizing an autosomal dominant inheritance. The index case father, mother, 4 siblings and 3 nephews totaling 9 individuals agreed to perform the customized assay. Assays based on AS-qPCR for the p.(Val142ILE) variant in the TTR gene and the p.(Met918Thr) variant in RET gene was developed. The index cases were used as positive controls. All tested subjects signed an informed consent form agreeing with the mutation research and were being accompanied by a geneticist. **Results** In the first family, among the 31 tested individuals the variant c.424G>A (p.Val142Ile) was detected in 8 (4 uncles, 4 cousins, and 1 nephew), always in heterozygous form. In the second family, among the 8 tested individuals, the variant c.2753T>C p. (Met918Thr) was not found in any tested family member, including the parents of the index case suggesting the occurrence of a de novo mutation. **Conclusion** The development of customized orthogonal tests for variants identified by MPS allows the evaluation of many family members providing important genetic information without the need for execution of a complex test (e.g. MPS) allowing to estimate the risk of developing clinical condition for family members and the provision of adequate genetic counseling.

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Validation of Massive Parallel Sequencing Workflows for Germline Variants: Method-Based or Analyte-Based Approach?

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Background: Method-based validation is the most appropriated approach to validate a massive parallel sequencing (NGS) workflow for a large panel of genes because of its big-data format. In this situation the comparison of two distinct NGS methods could be applied. However, classic validation protocols recommend the analyte-based approach to define the performance characteristics of a NGS method. So, NGS test results comparison with a gold standard or orthogonal method is indicated. Here, we describe an NGS workflow validation using both strategies, method-based and analyte-based approaches. **Methods:**Whole-blood from 16 volunteers was used. DNA was obtained using MagNA Pure 24 (Roche) and sequencing libraries were qualified/quantified using TapeStation4200 (Agilent). Test method included coding regions ±10bp flanking intronic sequences of 3921 genes enriched using Kappa HyperPlus (Roche) and SeqCap EZ inherited disease panel (Roche) and sequenced (2x75-bp) using NextSeq-500 (Illumina). Read alignment and variant calling were performed with Dragen Germline (P1) (Illumina), BWA enrichment (P2) (Illumina), and Varstation (P3) (Einstein). Reference method was performed using SureSelect Human All-Exon-V7 Exome (Agilent), SureSelectXT (Agilent) and sequenced (2x150-bp) using NextSeq-500. The same above-described bioinformatics pipelines were applied. Targeted regions shared by both enrichment systems were included in the analysis. For the method-based validation, SNP and INDELs called by the test method and by the reference method in each tested pipeline was compared and the number of variants and F-measure (harmonic mean between sensitivity and positive predictive value) were computed considering the reference method results as true. For the analyte-based validation, 60 SNVs called with minor allele frequency (MAF) of 40-60% were randomly chosen and tested by allele-specific qPCR and 60 indels with MAF of 35-65% were chosen sequentially and tested by fragment analysis in capillary electrophoresis in all 16 volunteers. The agreement and 95% CI between NGS and the orthogonal method were computed. **Results:**Target's mean coverage was 95X (83-99X) for the test method and 299.2X (274-375X) for reference method. For pipelines P1, P2, and P3, the test method called a median (min-max) of 6670 (6400-7169), 7296 (6964-7846) and 7343 (6923-7862) SNVs and 362 (329-385), 252 (229-295), 415 (342-460) indels, respectively. The reference called a median (min-max) of 6666 (6400-7138), 7279 (6959-7811) and 7277 (6949-7844) SNVs and 349 (328-367), 291 (266-319), 395 (350-420) indels, respectively. The median (min-max) F-Measure for pipelines P1, P2 and P3 were 98.67% (98.13-99.23%), 96.33% (95.70-97.01%), 96.15% (95.5-96.93%) for SNV and 77.03% (72.91-81.56%), 74.96% (70.59-79.6%), 68.74% (63.75-73.71%) for INDEL, respectively. The orthogonal methods generated interpretable/valid results in 49 of 60 SNV and 27 of 60 INDEL. All pipelines presented the same genotyping results. The observed agreement with NGS (considering the genotype) occurred in 833 of 845 instances (98.92%-95%CI: 97.98 - 99.44%) for SNV and in 459 of 463 instances (99.14%-95%CI 97.80-99.66) for INDEL. **Conclusion:**Method-based validation is more robust compared to the analyte-based validation because the increased number of tested variants. However, this approach also exacerbates divergence between both methods and lower agreement compared to the analyte-based validation was observed. Both approach together results in a strong NGS

and consistent NGS workflow validation.

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Performance Characteristics of Massively Parallel Sequencing of a Comprehensive Panel of Genes associated with Clinical Phenotypes on Samples Obtained from Self-Collected Buccal Swabs

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Introduction: There are advantages to using buccal swabs for clinical testing: they are relatively inexpensive, noninvasive and can be mailed out for self-collection and return-mailed for centralized testing. While peripheral blood is invasive, time-consuming, and more expensive. Here we evaluate the massively parallel sequencing of 3921 genes panel associated with inheritable diseases on self-collected buccal swabs samples and peripheral blood. **Methods**: DNA was extracted from 31 volunteers, 16 from self-collected buccal swabs (OCR-100, DNA Genotek) using Qiasymphony (Qiagen) and 16 from whole blood (WB) using MagnaPure24 (Roche). gDNA and sequencing libraries were qualified/quantified using TapeStation4200 (Agilent). Swab method included coding regions ±10bp flanking intronic sequences of 3921 genes enriched using Kappa HyperPlus Library Preparation Kit (Roche) and SeqCap EZ inherited disease panel (Roche) and sequenced (2x75-bp Mid Output V2 Reagent) using NextSeq-500 (Illumina) (estimated mean coverage-100X). Read alignment and variant calling were performed with BWA enrichment (BaseSpace Sequence Hub - Illumina). The test method was performed for the comparison with the sequencing using whole blood. The above-described bioinformatics pipeline was applied. Only targeted regions shared by both enrichment systems were included (3911 genes, 53980 genomic regions and 9957670 nucleotides). The VCF files of the control used on both runs obtained after the BWA-enrichment analysis were compared with VCF-eval (RTG-Tools) Positive percent agreement (true-positives/true-positives+false-negatives), analytical positive predictive value (true-positives/true-positives+false-positives) and the F-measure for heterozygous/homozygous variant calls were calculated considering the whole blood run as reference. **Result**s: The average of DNA extracted from swab and WB were 21,5±6 and 23,5±15 ng/uL, respectively. The average size of the library of the swab samples was bigger, 345,3±24,7 versus 311,4±19,7 bp. The target mean coverage was 96.8X (76%>50X) for the swab and 102X (88%>50X) for the WB. On both runs, the uniformity of coverage was the same (99.2%). The Percent Aligned Reads also were alike (99.57% vs 98.8%). The mean call for variants was 7135 SNVs and 245 indels on samples from the swab and 7217 SNVs and 241 indels on samples from WB. On the control analysis, the swab run called 7045 SNVs and 216 indels while the reference method called 70261 SNVs and 236 indels with 96.61% of precision, 96.6% of sensitivity, and F-measure of 96.61%. Both methods agreed in 7021 variants indicating a positive concordance of 96.93% for SNVs and 80,9% for indels, 215 SNVs, and 25 indels were called only by the swab and 195 SNVs and 45 indels were called only on the WB resulting in a technical positive predictive values for the test method of 96.94% for SNVs and 88.4% for indels. **Conclusio**n: Overall, these findings suggest that the WB samples yield a better sequencing quality. However, the self-collected samples obtained were suitable for clinical sequencing. The agreement with the reference method and technical positive predictive values were very good for SNVs and acceptable for indels calling (indels lead to more mismatches with capture probes than SNVs).

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Microbiology & Infectious Diseases

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Diagnostic Value of Serum pgRNA Detection in HBV-Infected Patients with Different Clinical Outcomes

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Background: Pregenomic RNA (pgRNA) is a direct transcription product of HBV covalently closed circular DNA (cccDNA) and plays important roles in viral genome amplification and replication. This study was designed to investigate whether serum pgRNA is a strong alternative marker for reflecting HBV cccDNA levels and to analyze the correlation between serum pgRNA, serum HBV DNA and hepatitis B surface antigen (HBsAg).

Methods: A total of 400 HBV-infected patients who received nucleos(t)ide analogs (NAs) therapy with different clinical outcomes were involved in this research. Case group included asymptomatic hepatitis B virus carrier (ASC), chronic hepatitis B (CHB), liver cirrhosis (LC), and hepatocellular carcinoma (HCC), 100 patients for each group. Recombinant plasmid for HBV pgRNA was constructed. The qRT-PCR was established and evaluated with standard recombinant plasmid. The methodological evaluation of qRT-PCR included sensitivity test, linear range test, specificity test, accuracy test, reproducibility test.

Results: The results showed that the level of HBV pgRNA had significant difference in these 4 groups. Serum pgRNA levels correlated well with serum HBV DNA levels and HBsAg levels (HBV pgRNA levels vs. HBV DNA levels, r=0.58, P<0.001; HBV pgRNA levels vs. HBsAg levels, r=0.47, P<0.001). In addition, we focused on the 108 HBV-infected patients with HBV DNA <500 IU/ml, it was surprising to find that 17.57% (13/74) of HBV pgRNA can be detected even when HBV DNA level was below 20 IU/ml.

Conclusion:HBV pgRNA levels in serum can be a surrogate marker for intrahepatic HBV cccDNA compared with serum HBV DNA and HBsAg. The detection of serum HBV pgRNA levels may provide a reference for clinical monitoring of cccDNA levels and the selection of appropriate timing for discontinuing antiviral therapy, especially when HBV DNA levels are below the detection limit.

Key words: real-time fluorescence quantitative PCR, hepatitis B virus, pregenome RNA, covalently closed circular DNA, clinical outcomes

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Characterization of Mutations across HBV Reverse Transcriptase in 499 CHB Patients in China

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Background: Mutations in hepatitis B virus reverse transcriptase are associated with nucleos(t)ide analogue resistance during long-term antiviral treatment. However, the drug resistance of many mutations in RT in patients with NA treatment has not yet been well illustrated.The objective of this study was to investigate the characterization of mutations in HBV RT and find some new drug-resistant mutations in NA-treated patients.

Methods: HBV RT sequences were analyzed by Sanger sequencing in 499 patients consisting of 285 treatment-naïve patients and 214 NA-treated patients.

Results: The difference of DNA sequence between genotype B and C in all patients was mainly attributed to synonymous mutations, while the difference of DNA sequence between treatment-naïve and NA-treated patients was mainly attributed to nonsynonymous mutations. Besides classic NA-resistant mutations such as rtM204V, rtN236T/I and rtA181V/T/I, mutations at rt299 position were more frequent in NA-treated patients than in treatment-naïve patients. In vitro phenotypic experiment, reduced replication capacity, resistance to lamivudine conferred by rtL229V and decreased sensitivity to entecavir conferred by rtL229V plus rtM204V were found.

Conclusion: The present study demonstrates a new NA-resistant mutation rtL229V which was helpful to evaluate the efficacy of antiviral drugs.

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Gut Microbiota Dysbiosis in Patients with Hepatitis B Virus-Induced Chronic Liver Disease Covering Chronic Hepatitis, Liver Cirrhosis, and Hepatocellular Carcinoma

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Background: The information regarding the effect of hepatitis B virus (HBV) infection on gut microbiota and the relationship between gut microbiota dysbiosis and hepatitis B virus-induced chronic liver disease (HBVCLD) is limited.

Methods: In this study, we aimed at characterizing the gut microbiota composition in the three different stages of hepatitis B virus-induced chronic liver disease patients and healthy individuals. Fecal samples and clinical data were collected from HBV-CLD patients and healthy individuals. The 16S rDNA gene amplification products were sequenced. Bioinformatic analysis including alpha-diversity, PICRUSt, etc. was performed.

Results: A total of 19 phyla, 43 classes, 72 orders, 126 families, and 225 genera were detected. The beta-diversity showed a separate clustering of healthy controls and HBVCLD patients covering chronic hepatitis (CHB), liver cirrhosis (LC), and hepatocellular carcinoma (HCC), and gut microbiota of healthy controls were more consistent, whereas those of CHB, LC and HCC varied substantially. The abundance of Firmicutes was lower, and Bacteroidetes was higher in patients with CHB, LC, and HCC than in healthy controls. Predicted metagenomics of microbial communities showed an increase in glycan biosynthesis and metabolism-related genes and lipid metabolism-related genes in HBVCLD than in healthy individuals.

Conclusion: Our study suggested that HBVCLD is associated with gut dysbiosis, with characteristics including, a gain in potential bacteria and a loss in potential beneficial bacteria or genes. Further study of CHB, LC, and HCC based on microbiota may provide a novel insight into the pathogenesis of HBVCLD as well as a novel treatment strategy.

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Identification and Characterization of *Plasmodium* Proteins in Saliva of Malaria Patients

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Background: Deregulation of host immune responses to inflammatory factors such as C-X-C motif chemokine 10 (CXCL10) and hosts angiogenic factors such as angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2) has been associated with malaria related mortality. However, detection of these factors in malaria patients requires the drawing of blood which is invasive, increases the risk of accidental blood-borne infections. There has been an increased interest in the use of saliva for the diagnosis of many infectious diseases including malaria. In this study, we assessed the plasma and saliva levels of biomarkers CXCL10, Ang-1 and Ang-2 in malaria patients and compare with non-malaria patients. **Methods**: This was a case-control study involving 213 participants: 119 malaria patients and 94 non-malaria patients. The study was conducted in the Shai-Osudoku District Hospital in Dodowa, Southeastern part of Ghana in the Greater Accra Region. The plasma and saliva levels of CXCL10, Ang-1 and Ang-2 of the study participants were measured using enzyme-linked immunoassay (ELISA) technique. Complete blood count (CBC) was measured with a Haematology autoanalyzer. Data were presented as median and interquartile range (IQR). A p-value of < 0.05 was considered significant. **Results**: Among 119 malaria patients, 44 (37.0%) were male and 75 (63.0%) were female whilst 27 (28.7%) male and 67 (71.3%) female represented the non-malaria patients and the ages of the participants ranges from 1-78 years, with a median ages of 29 (IQR 22-35) for non-malaria patients and 23 (IQR 17-31) for malaria patients (p<0.001).There was decreased plasma and saliva levels of Ang-1 (p<0.009) and increased plasma and saliva levels of CXCL10 (p<0.001) and Ang-2 (p<0.001) among malaria patients compared to non-malaria patients. **Conclusion**: This study provides the first evidence of detection of CXCL10, Ang-1 and Ang-2 in saliva of malaria patients.

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Multicenter Evaluation of Cefazolin MIC Results for *Enterobacteriaeceae* using CLSI Breakpoints on MicroScan Dried Gram Negative MIC Panels

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Background: A multicenter study was performed to evaluate the accuracy of cefazolin on a MicroScan Dried Gram-negative MIC (MSDGN) Panel when compared to a frozen CLSI/ISO broth microdilution reference panel.

Methods: A total of 450 *Enterobacteriaceae* clinical isolates were tested using the turbidity and Prompt® methods of inoculation during the efficacy phase at three U.S. sites. An evaluation was conducted by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI/ISO broth microdilution reference panel. A subset of 12 organisms was tested on MSDGN panels at each site during reproducibility. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 16-20 hours and read visually. All frozen reference panels were incubated at 35 ± 2°C for 16-20 hours and read visually. CLSI breakpoints (µg/mL) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 2 S, 4 I, ≥ 8 R.

Results: Reproducibility among the three sites were greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.

Essential, categorical agreement and categorical errors were calculated compared to MIC results from frozen reference panels for all isolates tested in efficacy and found in the following table.

Read Method	Essential Agreement (EA) %		Categorical Agreement (CA) %		Very Major Error (VMJ) %		Major Error (MAJ) %		Minor Error (MIN) %	
	P	T	P	T	P	T	P	T	P	T
WalkAway	97.1 (437/450)	97.1 (437/450)	97.6 (439/450)	98.0 (441/450)	1.6 (4/254)	1.2 (3/254)	3.4 (4/119)	0.0 (0/119)	0.6 3/450	1.3 6/450
autoSCAN-4	97.1 (437/450)	97.1 (437/450)	97.6 (439/450)	98.0 (441/450)	1.6 (4/254)	1.2 (3/254)	3.4 (4/119)	0.0 (0/119)	0.6 3/450	1.3 6/450
Visually	96.9 (436/450)	97.1 (437/450)	97.6 (439/450)	98.2 (442/450)	1.6 (4/254)	0.8 (2/254)	3.4 (4/119)	0.0 (0/119)	0.6 3/450	1.3 6/450

T = Turbidity inoculation method, P = Prompt inoculation method
*Calculation of CA and MIN excluding 1 well errors

Conclusion: This multicenter study showed that cefazolin MIC results for *Enterobacteriaceae* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria.

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Updated CLSI Meropenem Breakpoints for MicroScan Dried Gram Negative MIC Panels from a Multicenter Assessment of Enterobacteriaceae and Pseudomonas Aeruginosa

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Background: Updated US FDA/CLSI meropenem breakpoints were evaluated against data from a multicenter clinical study with *Enterobacteriaceae* and *P. aeruginosa* on a MicroScan Dried Gram-negative MIC (MSDGN) Panel. MIC results were compared to results obtained with frozen broth microdilution panels.

Methods: MSDGN panels were evaluated at four clinical sites by comparing MIC values obtained using MSDGN panels to MICs utilizing a CLSI broth microdilution reference panel. The study included 737 *Enterobacteriaceae* and *P. aeruginosa* clinical isolates tested using the turbidity and Prompt® methods of inoculation during the combined phases of efficacy and challenge. A subset of 11 organisms were tested on MSDGN panels at each site during reproducibility. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually. Read times for the MSDGN panels were 16-20 hours. Frozen reference panels were prepared and read according to CLSI methodology. FDA and CLSI breakpoints (µg/mL) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 1 S, 2 I, ≥ 4 R; *P. aeruginosa* ≤ 2 S, 4 I, ≥ 8 R.

Results: Essential and categorical agreement was calculated compared to frozen reference panel results. Results for isolates tested during efficacy and challenge with Prompt inoculation and manual read are as follows:

Read Method	Essential Agreement (EA) %	Categorical Agreement (CA) %	Very Major Error (VMJ) %	Major Error (MAJ) %
Enterobacteriaceae	95.5 (601/629)	98.4 (619/629)	0.0 (0/69)	0.0 (0/554)
P. aeruginosa	91.7 (99/108)	92.6 (100/108)	0.0 (0/25)	1.3 (1/77)

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

Conclusion: Meropenem MIC results for *Enterobacteriaceae* and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI interpretive criteria in this multicenter study.

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Bile Acids Inhibit Interferon-α Therapeutic Response through Impairing the Function of NK Cells in Chronic Hepatitis B Patients

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Background: Bile acids are involved in interferon signaling pathway in cell lines. However, the interrelationships between bile acids and interferon-α therapeutic response in chronic hepatitis B (CHB) is unclear.

Methods: Serum bile acid profile of 110 CHB patients was revealed by ultraperformance liquid chromatography-tandem mass spectrometry. The frequency of NK cells and the levels of IFN-γ and TNF-α were measured in 100 patients with chronic HBV infection.

Results: We demonstrated that CHB patients with high TBA levels had poorer response to pegylated interferon-alpha (PEG-IFN-α) therapy. Besides, we found that various bile acids, including taurocholic acid (TCA) and cholic acid (CA), were higher in non-response patients than that in sustained response patients received PEG-IFN-α therapy. Moreover, we found that taurocholic acid (TCA) inhibited the production of IFN-γ and TNF-α of NK cells in vivo and in vitro.

Conclusion: Bile acids inhibit IFN-α therapeutic response through impairing the function of NK cells, suggesting potential clinical therapeutic directions for reducing bile acids therapy before PEG-IFN-α treatment in CHB patients.

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Performance of the Elecsys Chagas Assay on the New Cobas e 801 Analyzer Versus Commercially Available Assays for Detection of Antibodies to Trypanosoma Cruzi

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Background: Chagas disease is caused by the protozoan *Trypanosoma cruzi* and affects 6-7 million people worldwide, predominantly in Latin America. The infection is transmitted congenitally or via insect vectors, blood transfusion, organ transplantation, and by ingesting contaminated food. Serology is the preferred method for diagnosis and blood donor screening. The Elecsys® Chagas assay (Roche Diagnostics) is

intended for the qualitative determination of *T. cruzi* antibodies in human serum and plasma. We evaluated the assay's performance on the cobas e 801 analyzer versus commercially available assays.

Methods: Measurements were conducted at the Fleury Diagnostic Medical Center (São Paulo, Brazil; May 2018-June 2018) using residual, pseudo-anonymized (i.e. no participant information was transferred/held by Roche Diagnostics) serum samples randomly collected during routine testing. The specificity of the Elecsys Chagas assay on the cobas e 801 analyzer was compared with two commercially available assays: Imuno-con Chagas (WAMA Diagnostica) and the Chagatest ELISA (Wiener Lab). Majority principle was applied among the three assays to define positive/negative results. Discrepant results were resolved by confirmatory testing at a central laboratory (Albert Einstein Hospital, São Paulo, Brazil) using the ARCHITECT Chagas chemiluminescent microparticle immunoassay (Abbott Laboratories) and immunofluorescence indirect testing.

Results: A total of 417 samples were evaluated (**Table 1**). Four samples were sent to Albert Einstein Hospital for further testing; one sample remained indeterminate based on a non-conclusive result. The Elecsys Chagas assay demonstrated a relative specificity of 100.00% (95% confidence interval [CI]: 99.08-100.00), versus 99.50% (95% CI: 98.20-99.94) for the Imuno-con Chagas assay (two false-positive results) and 98.22% (95% CI: 96.36-99.28) for the Chagatest ELISA (seven false-positive results).

Conclusion: The Elecsys Chagas assay on the cobas e 801 analyzer demonstrated high relative specificity for detecting *T. cruzi* antibodies in routine clinical use, while offering high automation and throughput.

Table 1. Relative specificity of the Elecsys Chagas assay on the cobas e 801 analyzer versus other commercially available assays in routine serum samples

Parameter	Elecsys Chagas	Imuno-con Chagas	Chagatest ELISA
Total samples tested, N	417	417	417
Non-reactive - confirmed indeterminate, n/N	1/400*	0/398	0/393
Non-reactive - confirmed positive, n/N	0/400	1/398	0/393
Negative, n	399	399	400
True negative, n	**399**	**397**	**393**
Reactive, n	17	19	24
False positive, n	**0**	**2**	**7**
Reactive - confirmed positive, n/N	17/17	16/19	16/24
Reactive - confirmed indeterminate, n/N	0/17	1/19*	1/24*
Reactive - confirmed negative, n/N	0/17	2/19	7/24
Relative specificity, % (95% confidence interval)**	**100.00 (99.08-100.00)**	**99.50 (98.20-99.94)**	**98.22 (96.36-99.28)**

*Indeterminate result according to further testing by Albert Einstein Hospital.
**Relative specificity was calculated as true negative/(true negative + false positive).

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Evaluation of Assay Performance in Cadaver (Organ Donor) Subjects for ADVIA Centaur® Infectious Disease Assays*

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Introduction: The demand for organ and tissue transplantation is rapidly growing worldwide. A patient risk in organ and tissue transplantation is infectious disease transmission resulting from the use of organs/tissue from an infected donor. The risk can be mitigated through infectious disease testing of donors using immunoassays. This study demonstrates the suitability of the ADVIA Centaur® CHIV†, aHCV§, HBsII, and aHBcT assays for cadaver (organ donor) testing. The study verified that the precision, specificity, and sensitivity of ADVIA Centaur® infectious disease assays are equivalent between cadaver subjects and living patients.

Objective: To establish the suitability of the ADVIA Centaur® CHIV, aHCV, HBsII, and aHBcT assays for cadaver testing. **Methods:** Cadaver serum (83 samples) collected within 24 hours of time of death was obtained from a commercial vendor. Serum (83 samples) was also drawn from healthy living patients. Three studies were performed. A CLSI 20 day precision study testing cadaver serum samples, a specificity study comparing 83 individual subject cadaver samples with 83 individual living patient serum samples, and a sensitivity study in which 50 cadaver samples and 50 living patient samples were spiked with high-titer infectious disease positive serum to target doses. The sensitivity study used acceptance criteria of +/- 25% average dose difference for spiked living patient samples vs. spiked cadaver subject samples as recommended by the Paul-Ehrlich-Institut 2008 "Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, Anti-HCV-Assays, HBsAg and Anti-HBc Assays for Use with Cadaveric Samples" guidance. For each study three reagent lots were evaluated and independently assessed against acceptance criteria. **Results:** Precision, Specificity and Sensitivity studies all generated results meeting acceptance criteria for all lots of all assays evaluated. **Conclusion:** The study indicates that the ADVIA Centaur® CHIV, aHCV, HBsII, and HBcT assays are acceptable for use in routine cadaver (organ donor) testing.*Products with cadaveric claims are under development and not available for sale. Product availability may vary from country to country and is subject to varying regulatory requirements.†Assay developed, manufactured, and sold by Siemens Healthcare Diagnostics Inc. for Ortho Clinical Diagnostics, Inc. and Grifols Diagnostic Solutions Inc.§For outside the U.S., assay developed, manufactured, and sold by Siemens Healthcare Diagnostics Inc. for Ortho Clinical Diagnostics, Inc. and Grifols Diagnostic Solutions Inc.

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A Host-Response Signature Based on TRAIL, IP-10 and CRP Addresses Antibiotic Misuse Driven by Diagnostic Uncertainty

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Background:

Children arriving to the emergency department with fever without source (FWS) or respiratory tract infection (RTI) often present a diagnostic challenge. Here we evaluated whether clinical uncertainty drives antibiotic misuse and if a novel host-response signature comprising TRAIL, IP-10, CRP (MeMed Ltd) that distinguishes viral from bacterial infection could potentially reduce uncertainty, improve diagnostic accuracy and support better informed antibiotic decisions.

Methods:

We performed multinational prospective evaluation at pediatric emergency departments (ED) in Germany and Italy ("AutoPilot-Dx"; grant #701088). Infection etiology was determined by unanimous decision of 3 independent experts. Managing ED physicians were asked to estimate their certainty if the patient had a viral or bacterial infection. Association between clinical uncertainty and antibiotic misuse was evaluated. The signature outputs a score between 0 and 100, accorded 5 score bins. Diagnostic performance of the signature vs managing physician was assessed across all patients and those with diagnostic uncertainty, according to pre-determined cutoffs.

Results:

A total of 732 children were included in the final analysis cohort (628 viral, 104 bacterial). Managing physicians reported diagnostic uncertainty for 537 of the 732 patients (73%). Overuse and underuse antibiotic rates were higher in the uncertain as compared to certain sub-cohort (34.0% vs 16.8%, 10.0% vs 5.9%, respectively). The likelihood ratio for bacterial infections exhibited a significantly increasing trend with score ($P<0.001$; figure 1). A potential reduction in antibiotic overuse of 3.3-fold (from 30% to 9%) and underuse of 1.3-fold (from 9% to 7%) was observed. In the uncertain sub-cohort, the potential reduction in antibiotic overuse and underuse was higher at 3.8-fold (34% to 9%) and 1.7-fold (10% to 6%), respectively.

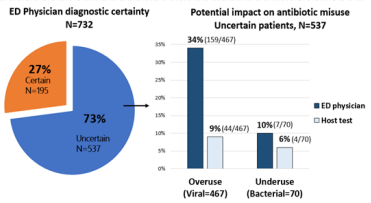
Conclusions:

ED physician diagnostic uncertainty drives antibiotic misuse among children with RTI or FWS. The TRAIL/IP-10/CRP signature shows high performance for distinguishing between bacterial and viral infections, especially for patients with diagnostic uncertainty, and may help reduce antibiotic misuse.

Bacterial or viral infection (N=732)

	Patients n (%)	Bacterial n (%)	Viral n (%)	LR (95% CI)
90 ≤ score ≤100	77 (10.5)	73 (94.8)	4 (5.2)	110.20 (40.71-298.28)
65 < score <90	45 (6.1)	16 (35.6)	29 (64.4)	3.33 (1.84-6.03)
35 ≤ score ≤ 65	72 (9.8)	9 (12.5)	63 (87.5)	0.86 (0.44-1.71)
10 < score <35	151 (20.6)	4 (2.6)	147 (97.4)	0.16 (0.06-0.44)
0 ≤ score ≤10	387 (52.9)	2 (0.5)	385 (99.5)	0.03 (0.01-0.12)
Total	100.0	104	628	

A.



B.

Figure 1. The likelihood ratio (LR) for bacterial infection increases as the score increases (A). ED managing physician overuse and underuse rates were compared to those of the signature across patients about whom the ED managing physician was clinically uncertain (B). ED physician overuse was defined as viral patients receiving antibiotics, underuse as bacterial patients not receiving antibiotics or receiving delayed treatment. The signature's potential misguidance of treatment was defined according to pre-defined CE-IVD cutoffs: overuse was viral patients with a score over 65, indicative of a bacterial infection (false positives), underuse as bacterial patients with a score under 35, indicative of viral infection (false negatives). Equivocal results corresponding to scores 35-65 do not provide diagnostic information and so potential antibiotic misguidance in these cases was defined according to the ED physician's treatment.

A-240

Performance Evaluation of the ADVIA Centaur and Atellica IM CMV IgM Assays*

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Background: Siemens Healthineers is developing a fully automated cytomegalovirus IgM (CMV IgM) assay for qualitative detection of IgM antibodies to cytomegalovirus (CMV) in serum and plasma. Antibodies to CMV IgM are used as an aid in the diagnosis of recent or current CMV infection in individuals for which a CMV IgM test was ordered. The automated Atellica® and ADVIA Centaur® CMV IgM Assays* are chemiluminescent magnetic microparticle-based immunoassays.

Methods: The performance of the prototype CMV IgM assay was evaluated on Atellica® Immunoassay Analyzers and ADVIA Centaur® Systems. Negative agreement was evaluated with 100 pregnancy and 54 pediatric samples that were collected prospectively. Positive agreement was determined by testing 64 samples that were positive by BioMerieux VIDAS and Siemens IMMULITE® 2000 CMV IgM assays. The results were assessed based on index values as reactive (index ≥1.0) and nonreactive (index <1.0). Precision was evaluated by testing seven samples with index values spanning the assay range in one run per day for 5 days on the Atellica Immunoassay Analyzer and ADVIA Centaur Systems. Five seroconversion panels and one mixed titer panel from commercial vendors were also tested and the results were compared to the BioMerieux VIDAS CMV IgM assay. Interference was evaluated per CLSI EP37.

Results: Negative percent agreement for the 154 prospective pediatric and pregnancy samples was ≥97%. Positive percent agreement for the 64 CMV IgM positive samples was ≥98%. Overall clinical agreement between CMV IgM on the Atellica Immunoassay Analyzer and ADVIA Centaur Systems was ≥99%. Good precision with repeatability and within-lab %CV of <6.0% and <8.0% respectively, for samples yielding index values between 0.4 and 8.0, was demonstrated. The assay showed equivalent seroconversion sensitivity to the BioMerieux VIDAS CMV IgM assay, where all negative panel samples were nonreactive, and all positive panel samples were reactive. No clinically significant differences were observed for common interferents at the levels tested per CLSI EP37.

Conclusions: These study results demonstrate good performance of the prototype CMV IgM assay on the Atellica Immunoassay Analyzer and ADVIA Centaur Systems.

*Under development. Not available for sale. The performance characteristics of this product have not been established. Product availability will vary from country to country and will be subject to varying regulatory requirements.

A-242

Performance of an Influenza A, Influenza B, and RSV Multiplex Full Process Molecular Control across Assay Systems

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Background: Molecular assays for seasonal flu are an important diagnostic tool to identify infection and expedite patient treatment. Clinical labs use reverse transcription-polymerase chain reaction (RT-PCR), and other nucleic acid amplification tests to detect influenza and respiratory syncytial virus (RSV) RNA with high sensitivity and specificity. These tests require routine QC monitoring, and stable, multiplexed, reproducibly manufactured, low positive reference materials can greatly improve the effectiveness and efficiency of QC. SeraCare has developed a multiplexed FluA, FluB and RSV reference material that mimics low positive patient specimens and is compatible with leading assays.

Methods: SeraCare developed the ACCURUN® Multiplex Flu/RSV reference material utilizing proprietary AccuPlex® recombinant technology. The reference material contains the entire genomic RNA from influenza A(H1N1)pdm09 lineage, Influenza B/Victoria lineage, and ~75% of the genomic RNA sequences from RSV RSS-2 subtype A strain. AccuPlex recombinant viruses require extraction,but are replication-deficient and heat-treated. and so are non-infectious. The recombinant viruses for FluA, FluB and RSV were mixed together and digital PCR was used to verify that each virus was present in the mix at the same copies/mL concentration. The mixture was then serially diluted in a viral transport media. The dilution series were assigned concentrations based on virus-specific droplet digital PCR. The dilution series was subsequently tested using Cepheid Xpert® Xpress Flu and RSV and Cepheid Xpert® Xpress Flu assays at two different laboratories, as well as on the BioFire® FilmArray® Respiratory Panel, to assess assay sensitivity. Based on the results, a prototype low positive reference material was manufactured and multi-site, multi-assay testing is ongoing.

Results: Testing at both sites resulted in 100% positivity for the Cepheid Xpert Xpress Flu, or Xpert Xpress Flu and RSV when FluA, FluB and RSV were present at concentrations of at least 4000 copies/mL. Positivity was greater than 95% on the Cepheid assays when the analytes were present at concentrations as low as 800 copies/mL. Samples tested on the BioFire Respiratory panel were also detected as positive at a concentration of 800 copies/mL. Therefore, the lower limit of detection for these assays can be estimated at approximately 800 copies/mL. Manufactured QC reference materials will be targeted at 3-5 times this lower limit to effectively challenge assay performance.

Conclusions: SeraCare has developed a stable, non-infectious, multiplexed, full process reference material for FluA, FluB and RSV molecular assays. The material was targeted to be low positive for each of the three analytes, which is important for rigorous and effective QC monitoring of test performance. The material can also be used for assay validation, competency evaluation, and reagent lot release testing.

A-243

Eliminating the Cold-Chain Transport of Samples for Bacterial Testing: A Laboratory Study

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Background: Clinical microbiology laboratories are increasingly being centralized to help contain laboratory costs. This has resulted in the need for rapid cold-chain transport of samples for bacterial testing from the source to the laboratory. This rapid cold-chain transport is expensive. Although the transports are not monitored, breaks in the cold-chain occur frequently. These breaks adversely affect the organisms in the transported samples, especially if the number of organisms on the swab is low ($\leq 10^1$). We have developed a transport system that eliminates the need for rapid cold-chain transport of samples for bacterial testing (LabReady®). LabReady enriches the sample in transport. We tested this new system against the current standard (BBL Culture Swab™ Plus) using group B streptococcus (GBS).

Methods: Seven strains of GBS (6 ATCC and 1 clinical, composed of 6 serotypes, hemolytic and non-hemolytic) were individually grown and dilutions were prepared to achieve starting GBS counts (on each swab) to range from 1 to 7 cfu. The dilution with desired GBS concentration was used as sample source for the BBL Culture Swab™ Plus and the LabReady® GBS transport systems. Statistical comparisons were performed using the Fischer exact test.

Results: 100% of samples collected and transported with the LabReady GBS transport system were GBS positive after 24 h at 2°C (n=42), or 24 h at 37°C (n=24). These samples remained GBS positive out to 14 d after collection and continued storage at 2°C (n=42) or 37°C (n=24). 78% of samples collected and transported with the BBL Culture Swab Plus system were GBS positive after 24 h of refrigeration (4-8°C) (n=18, p <.0063 vs LabReady GBS). Among the samples collected and transported with the BBL Culture Swab Plus system, one was positive for GBS (n=4) after 24 h at room temperature (25°C) and none of the samples were GBS positive after 37°C for 24 h (n=4, p=0 vs LabReady GBS). After 24 h, 96h and 14 d of incubation, the GBS cultures from LabReady GBS transport system had concentrations of $\geq 1x10^9$ cfu/ml.

Conclusion: Low counts of GBS inoculum were lost in transport simulating environmental temperatures ranging from 4 to 37°C using the current standard BBL Culture Swab Plus system but not with the LabReady GBS system. The LabReady GBS helps eliminating the false negative results, especially when the starting bacterial count in sample is low.

A-244

Development of a Single Tube, Multi-Analyte Respiratory Syndrome Run Control

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Objective: This study details the development and validation of a single tube multi-analyte respiratory quality control quality across three assays on two instrument platforms.

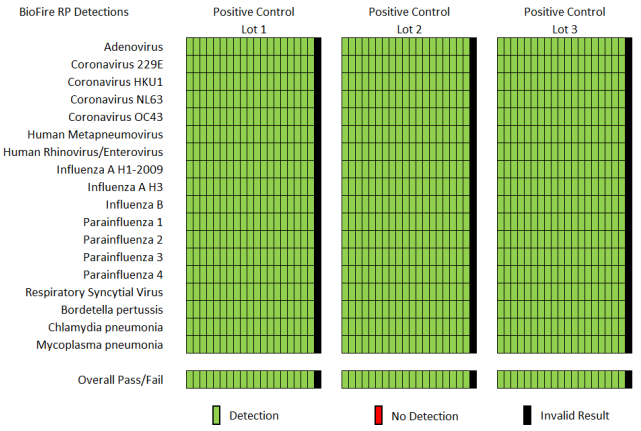
Background: Respiratory syndromes disproportionately impact the young, the old, and the immunocompromised where faster, more accurate test results are needed the most. To address this need, PCR-based molecular diagnostics has risen in usage in clinical laboratories across the US. With the implementation of IQCP, access to multi-analyte quality controls that reliably mimic specimen samples is vital to clinical laboratory performance. We set out to develop a single tube, 22-analyte respiratory syndrome control that meets the QC needs of laboratories running PCR-based assays.

Methods: We created a mixture of 22 viral or microbial analytes, each value assigned with an absolute copy concentration using digital-droplet PCR, and traceable to a higher order standard. Analyte stability was determined through long-term shelf-life, accelerated stability, open-vial, as well as freeze-thaw studies.

The product was developed and tested as a low control. Experimental determination of the optimal concentration of each analyte, to challenge the assay systems as well as minimize laboratory cross contamination, was performed on prototype mixtures using the BioFire® FilmArray® Respiratory (RP) Panel and the BioFire® FilmArray® Respiratory 2 (RP2) Panel.

Validation of mixture performance was conducted on the BioFire RP & RP2 Panels across three lots of low control (n=60 & n=60) and the Cepheid Xpert® Xpress Flu/RSV using a single lot (n=2).

Results: Performance studies demonstrated concordant analyte detection across multiple lots, assays, runs, operators, and instruments. Stability of the mixture was determined to be 18 months, stored at -20°C. A representative sample of the BioFire RP Panel data is below:



Conclusion: The Exact Diagnostics Respiratory Run Control (RPPOS), demonstrates consistent stability and performance needed for a single tube, multianalyte run control.

A-245

Analytical Performance of the Elecsys Anti-HAV II Immunoassay on the cobas e 601 Analyzer in Samples from US Individuals

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Background: Approximately 30% of US individuals may have hepatitis A virus (HAV) IgG antibodies, which could indicate previous infection. HAV cannot be differentiated from other viral hepatitis strains using clinical/epidemiologic factors alone. Accurate serological tests to aid diagnosis and determine antibody response in vaccine recipients are required. We evaluated the analytical performance of the Elecsys® Anti-HAV II immunoassay (Roche Diagnostics; detects total antibodies [IgG and IgM]) and conducted a method comparison versus the Elecsys Anti-HAV assay. Major refinements in the 2nd generation assay include: pre-wash, improved on-board stability and cut-off based qualitative interpretation.

Methods: Precision of the Elecsys Anti-HAV II assay (cobas e 601 analyzer; CLSI-EP05-A3 criteria) was evaluated using two Anti-HAV II PreciControl materials and four human serum pools (HSPs; reactive; non-reactive; three replicates per run, two runs per day for five days; three US laboratories). Repeatability, intermediate precision and reproducibility were calculated. Seroconversion sensitivity (versus Elecsys Anti-HAV) was evaluated (4 sample panels; 8-15 samples each). HAV prevalence was assessed in apparently healthy volunteers using samples from two collection sites (Eastern and Western USA). Method comparison (versus Elecsys Anti-HAV) was conducted using remnant and retrospective serum/plasma samples across several cohorts (hospitalized; increased risk for hepatitis; symptomatic; characterized acute HAV; pediatric [4-21 years]; routine HAV testing; vaccinated [VAQTA, HAVRIX or TWINRIX], samples collected pre- and post-vaccination). Positive/negative percentage agreements (PPA, NPA) with 95% confidence intervals (CI) were determined.

Results: Coefficients of variation (CV)/standard deviation (SD) for repeatability, intermediate precision and reproducibility across sites, and method comparison results are presented; see **Table**. Seroconversion sensitivity was equivalent for the two assays across all 4 sample panels. HAV prevalence was higher in samples collected from Western (215/400 [53.75%]) versus Eastern (118/400 [29.50%]) USA.

Conclusion: The Elecsys Anti-HAV II assay demonstrated good analytical performance and high agreement with the Elecsys Anti-HAV assay.

Table. Elecsys Anti-HAV II assay analytical performance and method comparison vs Elecsys HAV assay

Precision			
Analyte reactivity	**Repeatability**	**Intermediate precision**	**Reproducibility**
Reactive (SD)	0.004-0.013	0.009-0.023	0.014-0.024
Non-reactive (CV%)	0.9-2.1	1.4-2.6	2.2-2.7
Method comparison			
Cohort	**Samples, n**	**PPA† % (95% CI)‡**	**NPA† % (95% CI)‡**
Routine HAV testing	200	100 (96.03-100.00)	94.50 (88.40-97.95)
Hospitalized	200	98.21 (90.45-99.95)	97.22 (93.04-99.24)
Increased risk for hepatitis	206	100 (96.95-100.00)	94.25 (87.10-98.11)
Symptomatic	220	100 (97.18-100.00)	96.70 (90.67-99.31)
Characterized acute HAV	75	100 (94.48-100.00)	100 (69.15-100.00)
Pediatric	60	100 (91.59-100.00)	77.78 (52.36-93.59)
OVERALL*	**961**	**99.80 (98.90-99.99)**	**95.21 (92.83-96.97)**
Vaccinated (pre- and post-samples, all vaccines)	**49**	**100 (92.75-100)**	**100 (92.75-100)**

*Excludes samples from the vaccinated cohort
†Calculated based on a cutoff index of 1.0 for the Elecsys Anti-HAV II assay and a borderline range of 18.0-22.0 IU/L for the Elecsys Anti-HAV assay. Borderline samples were retested in duplicate to confirm measurement
‡Clopper-Pearson exact 2-sided 95% confidence intervals

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Evaluation of Adenosine Deaminase (ADA) in Serum, Pleural, Pericardial, Peritoneal, and Cerebrospinal Fluids using the Diazyme Assay on the Roche c501 Chemistry Analyzer

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Background: Adenosine deaminase (ADA) plays a role in cell-mediated immune response and can appear in many body fluid types during infectious and inflammatory states. Measurement of pleural fluid ADA may provide supportive information in the evaluation of suspected Mycobacterium tuberculosis, particularly in settings that do not have access to molecular or culture-based methods. An automated research-use-only (RUO) assay for ADA is commercially available. The objective of this study was to evaluate the performance characteristics the Diazyme ADA assay for serum, pleural, pericardial, peritoneal, and cerebrospinal fluids using the Roche c501 chemistry analyzer. **Methods:** Method comparison for serum and pleural specimens was

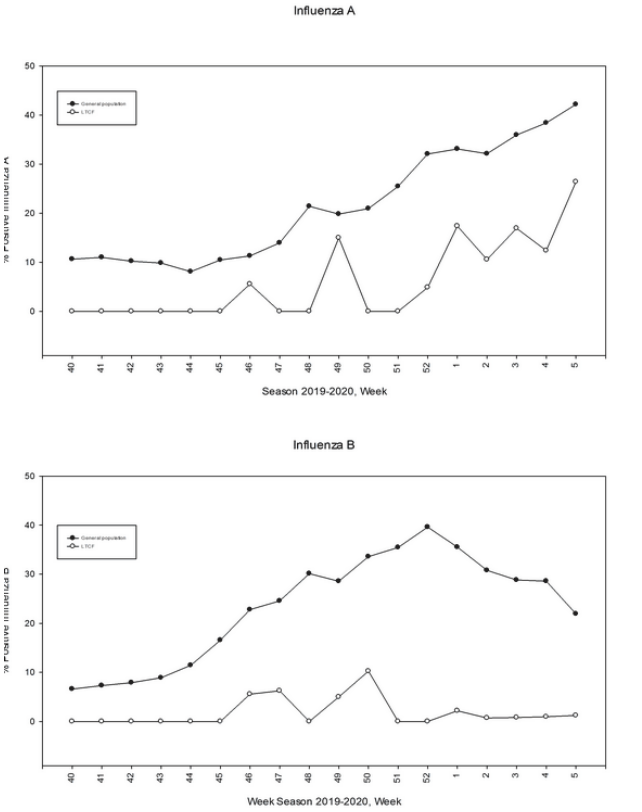
performed by analyzing samples onsite and at external facilities. Linearity studies were performed with ADA spiked into serum, peritoneal, and cerbrospinal fluids; endogenous ADA samples were used for pericardial and pleural fluids. Recovery studies used spiked samples for all fluid types. Imprecision was evaluated using 2 concentrations of quality control (QC) material. For intra-assay studies, 20 replicates of each QC level were analyzed in one run. Inter-assay precision was assessed by analyzing 40 replicates of QC over 10 days, 4 replicates per day. Limit of Detection (LoD) was assessed by analyzing instrument responses for 10 replicates of saline and 3 replicates of low concentration patient samples. Interference was evaluated for serum using varying concentrations of hemolysate, intralipid, and bilirubin (conjugated and unconjugated). **Results:** Method comparison for serum samples demonstrated a slope of 1.053 (bias 4.4%). Pleural specimens demonstrated a slope of 1.046 (2.6%). Linearity was verified for all fluid types. Spiked recovery ranged from 94.3-109.3% across fluids. Imprecision across fluid types was 0.6-4.1%CV (intra-assay) and 1.1-4.6%CV (inter-assay). LoD of 1.00 U/L was verified. Hemolysis and lipemia did not show significant interference (<10%) at concentrations tested. Significant negative interference was detected with increased concentrations of conjugated and unconjugated bilirubin. **Conclusions:** The Diazyme ADA assay demonstrated acceptable analytical performance for all fluid types evaluated and met manufacturer criteria where applicable. Bilirubin ≥ 16 mg/dL showed negative interference with the ADA assay.

A-249

Monitoring Influenza A and B in Long-Term Care Facilities: Flu Season 2019-2020

R. Khoury, A. Gandhi, P. Patel, H. Massey, D. Gudaitis, P. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

Context: Influenza is the most common infectious disease, causing over 11,000 cases and over 1,200 deaths in January 2020. It is caused by influenza virus A, B, or C. Flu is a serious condition in Long-Term care Facilities (LTCF) where most of the residents are elderly, frail, disable, on multiple medications; they are at greater risk of developing serious complications compared to young healthy adult. Preventing transmission of influenza include: vaccination, testing, infection control, and appropriate treatment. **Design:** 943 nasal swabs were collected from residents in LTCF from the October 2019 (week 40) to February 2020 (week 5) of the 2019-2020 season. Tests were done using Solana Influenza A+B Assay for the detection and differentiation of influenza A and influenza B viral RNA in nasal and nasopharyngeal swabs and rapid influenza diagnostic test; data was separated weekly staring week 40 2019 to week 5 2020. Results were compared to national influenza surveillance data. Statistical analysis was done using Analyse-it. **Results:** 124 patients and 16 patients were positive for influenza A and influenza B respectively; influenza A was most prevalent in all weeks tested with peak in week 5 reaching 26.4% of the LTCF patients tested, and it was the highest week on national level reaching 42.2% of patients tested. Although some of the patients tested positive from the LTCF were hospitalized but no death has been reported to our knowledge due to influenza. **Conclusion:** The combination of enforcing vaccination to all residents and workers against the flu every year, early detection, isolation and administration of antiviral chemoprophylaxis to all residents as soon as they have an outbreak will prevent the complication and spreading, and the availability of infection control program give the residents in LTCF the benefit of lower influenza incidence, hospitalization, complication, and mortality compared to national published data.



A-253

Performance Evaluation of the ADVIA Centaur® aHBe II Assay

T. Bori-Sanz[1], D. Mañe[1], E. Saxton[2], J. San Roman[2], Z. Bostanian[3], E. Schiff[4], R. Christenson[5]. *[1]Biokit Research & Development SLU, Barcelona, Spain, [2]Siemens Healthcare Diagnostics Inc, Tarrytown, NY, [3]Research and Development Institute, Van Nuys, CA, [4]University of Miami, Miami, FL, [5]University of Maryland, Baltimore, MD*

Background: Hepatitis B is a life-threatening liver infection caused by the Hepatitis B virus (HBV), a major global health problem with an estimated prevalence of 0.7% - 6.2% of the population. It is estimated that approximately 257 million individuals are chronically infected and at high risk of illness and death from liver cirrhosis and hepatocellular carcinoma. During viral hepatitis infection many serological markers appear. The presence of the Hepatitis B envelope antigen (HBeAg) is associated with high levels of HBV viremia associated with high levels of infectivity. The presence of Hepatitis B e antibody (Anti-HBe) represents the host response to the HBeAg presence and is present during the immune-control and immune-escape phases. The presence of anti-HBe (HBeAg positive seroconversion) with a decline in HBV replication and normalization of ALT levels may indicate HBV classification stage (acute, chronic, early recovery, recovery, immune, recovered). The presence of anti-HBe can be used to guide treatment as well as monitor treatment response. The ADVIA Centaur® anti-HBe II* assay is a fully automated 2-step competitive immunoassay that uses chemiluminescent technology for the qualitative detection of Hepatitis e antibody in human adult and pediatric serum or plasma (EDTA and lithium heparin) samples. The aim of this study was to evaluate the performance of the prototype assay on the ADVIA Centaur® XP, XPT and CP Immunoassay Systems. **Methods:** Samples were collected at four US sites and tested at three US sites. Individuals met criteria for inclusion by being at risk for Hepatitis B or experiencing signs and symptoms of the disease. A total of 1776 samples from subjects in various disease states as confirmed by characterization of the samples using commercial assays (acute, chronic, early recovery, recovery, immune natural infection, recovered, and not previously infected) was analyzed. Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the aHBeII assay were assessed against the VITROS® Anti-HBe assay. In addition to method comparison, performance characteristics evaluations included reproducibility using a 4-member panel that was assayed in replicates of 4 with 2 runs per day, over 5 days for each lot (n = 240 for each sample), and seroconversion,

using 3 subjects with bleed-days ranging from 70 to 84 days. **Results:** Evaluation of patient samples using the ADVIA Centaur anti-HBe II assay on the ADVIA Centaur XP system indicated a PPA% of 97.6% (95%CI 94.0% - 99.1%) and an NPA% of 99.0% (95% CI 98.4% - 99.4%) compared to the VITROS Anti-HBe assay results. The assay demonstrated good reproducibility with CV of 2.3-8.3%. The commercially available HBV patient seroconversion panels were tested using the ADVIA Centaur anti-HBe II assay to determine the seroconversion sensitivity. There were no differences in bleed days when compared to VITROS Anti-HBe assay. Similar performance characteristics were obtained on the ADVIA Centaur XPT and the ADVIA Centaur CP system. **Conclusions:** Study results demonstrate acceptable performance characteristics of the ADVIA Centaur anti-HBe II assay on the ADVIA Centaur XP, XPT and CP systems.*Under development. Not commercially available. Future product availability may vary from country to country and will be subject to varying regulatory requirements.

A-254

Analytical Performance of the VITROS® Immunodiagnostic Products Anti-HTLV I/II Assay*

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Objective: This study was designed to assess the analytical performance of the VITROS Immunodiagnostic Products Anti-HTLV I/II assay (VITROS Anti-HTLV)* on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600/ XT 7600 Integrated Systems. The assay detects antibodies to HTLV Types I and II. **Methods**: Antibody detection in VITROS Anti-HTLV is achieved using recombinant HTLV antigens coated onto the well. Sample is added to the coated wells in the first stage of the reaction and HTLV antibody from the sample is captured. After washing, HRP conjugated recombinant HTLV antigens are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. The assay cut-off for VITROS Anti-HTLV is 1.00; values above the cut-off are Reactive for HTLV antibodies and values below 1.00 are Non-reactive. Analytical sensitivity was evaluated using two commercially available performance panels and 25 fresh spiked patient samples. Analytical specificity was assessed consistent with CLSI EP7 and also by testing patient samples known to include potentially interfering substances. Assay reproducibility was assessed consistent with CLSI EP05 using two reagent lots with a 7 member panel. VITROS Anti-HTLV was tested for equivalence in serum and EDTA, Heparin and Sodium Citrate plasmas - including serum and lithium heparin separator tubes. College of American Pathologists (CAP) Viral Marker series 3 (VM3) was tested in triplicate to confirm compatibility with VITROS Anti-HTLV I/II. **Result**s: Results for all commercial panel samples were concordant with the package insert on each VITROS System. Twenty-five fresh spiked samples each generated Reactive results. Potential interfering substances [1000 mg/dL hemoglobin, 40 mg/dL bilirubin, 3510 ng/mL biotin, 1500 mg/dL triglycerides, 15 g/dL total protein, 2130 mg/dL IgG, 1242 mg/dL total cholesterol] were tested and shown to not impact test results. Other disease states, including but not limited to HIV, Hepatitis A, B and C, as well as samples from pediatric (2 to 17 years of age) and pregnant patients were shown to not impact test results. Within-run imprecision for 5 reactive samples ranged from 0.18 to 2.69 %CV. Total imprecision for 5 reactive samples ranged from 3.30 to 6.24 %CV. The calibration was shown to be stable for 91 days. Results collected in serum and EDTA, heparin and sodium citrate, including separator tubes, was shown to generate clinically equivalent results. VITROS Anti-HTLV I/II results for CAP VM3 were consistent with survey results. **Conclusio**n: The VITROS Anti-HTLV I/II assay* demonstrates excellent analytical performance in the detection of HTLV I/II antibodies. *Under development.

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Performance of the VITROS® Immunodiagnostic Products Anti-HTLV I/II Assay* in Two Clinical Laboratories

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Objective: This study was designed to assess the clinical performance of the VITROS Immunodiagnostic Products Anti-HTLV I/II assay (VITROS Anti-HTLV)* on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600/ XT 7600 Integrated Systems. In addition, assay performance for VITROS Anti-HTLV I/II was compared to the Abbott ARCHITECT rHTLV-I/II assay (ARCHITECT rHTLV-I/II). The assay detects antibodies to HTLV Types I and II. **Methods**: Antibody detection in VITROS Anti-HTLV is achieved using recombinant HTLV antigens coated onto the well. Sample is added to the coated wells in the first stage of the reaction and HTLV antibody from the sample is captured. After washing, HRP conjugated recombinant HTLV antigens are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. The assay cut-off for VITROS Anti-HTLV is 1.00; values above the cut-off are Reactive for HTLV antibodies and values below 1.00 are Non-reactive. Testing was performed at two sites using two reagent lots on a VITROS ECi/ECiQ Immunodiagnostic System, VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System. Clinical sensitivity was evaluated using frozen patient samples. 434 HTLV positive samples were tested in triplicate on both VITROS Anti-HTLV and ARCHITECT rHTLV-I/II. Clinical specificity was evaluated using fresh samples from 5096 HTLV negative blood donors and frozen samples from 204 HTLV negative hospitalized patients. Samples were tested in singleton on each method. Samples that generated an initial result above the assay cut-off were retested in duplicate to determine final interpretation of result for that sample. Data were analyzed to calculate the clinical sensitivity and clinical specificity for both methods. Discordant results were resolved by performing confirmatory testing with independent reference methods. **Results**: The sensitivity of VITROS Anti-HTLV was 100.0% (434/434, 95% CI: 99.2-100.0%) compared to 99.8% for ARCHITECT rHTLV-I/II (433/434, 95% CI: 98.7-100.0%). Specificity in the blood donor population for VITROS Anti-HTLV was 99.94% (5093/5096, 95% CI: 99.83-99.99%) compared to 99.84% (5088/5096, 95% CI: 99.69-99.93%) for ARCHITECT rHTLV-I/II. Overall specificity for VITROS Anti-HTLV was 99.92% (5296/5300, 95% CI: 99.81-99.98%) compared to 99.83% (5291/5300, 95% CI: 99.68-99.92%) for ARCHITECT rHTLV-I/II. **Conclusion**: The VITROS Anti-HTLV I/II assay* demonstrates excellent clinical sensitivity and specificity. VITROS Anti-HTLV I/II is intended to be used as an aid in diagnosis of HTLV infection and to screen donors of blood, blood components, cells, tissue and organs for the presence of HTLV infection. *Under development.

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Use of Accuplex Recombinant Virus Technology to Produce a Non-Infectious, Whole Process Reference Material for 2019 nCoV (Wuhan)

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Background: An outbreak of a novel coronavirus occurred in Wuhan, Hubei Province, China in late 2019. Since that time, more than 40,500 cases of the virus have been confirmed worldwide, with the vast majority of those in mainland China. The virus is genetically related to SARS. It causes respiratory illness with fever, cough, and difficulty breathing and can result in pneumonia, respiratory failure, and death. The World Health Organization (WHO) declared the situation to be a public health emergency. To slow the global spread of this virus, diagnostic testing is extremely important so that those infected can be properly quarantined and their contacts monitored. Diagnostic test developers are quickly working to design, manufacture and validate molecular diagnostic assays for emergency use. This development requires non-infectious, stable, and reproducibly manufactured positive reference materials. SeraCare has developed AccuPlex 2019-nCoV Reference Material to meet this need.

Methods: The 2019-nCoV virus has a single stranded RNA genome of approximately 29.9 Kb. Recombinant viruses were designed to contain regions of the genome (NC_045512.2) targeted by published amplified nucleic acid diagnostic tests. Regions include portions of ORF1a and RdRP regions, as well as the entire E, M, ORF7a, ORF8 and N genes. The constructs were used for in vitro RNA transcription, and RNA was introduced into BHK-21 cells where recombinant Sindbis virus particles were assembled. The recombinant Sindbis virus vector system produces viral particles that are replication defective because they lack genes for structural proteins. Additionally, dividing the nCoV sequences among multiple recombinant viruses, engineering STOP codons into the constructs to prevent production of functional proteins, and heat treating to inactivate the viruses all add to the safety of the reference material. **Results:** TaqMan digital PCR assays based on published literature (for example primers and probes published by the US CDC, Tib-Molbiol, Berlin, Germany and the Chinese Center forDisease Control and Prevention, Beijing, China) were used to quantitate the recombinant viruses. Viruses were mixed at equimolar concentrations and diluted in viral transport media. The reference material is undergoing testing using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel as well as other real time PCR assays in development, and results will be presented.

Conclusions: SeraCare has developed a stable, well-characterized full-process control for molecular assay targeting 2019-nCoV (Wuhan). This reference material is

enabling diagnostics for emergency use to meet regulatory testing requirements and be released for use more rapidly,and is helping laboratories to validate tests and train technicians to ensure preparedness.

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Performance Evaluation of the BD MAX™ Bacterial Enteric Panel: The Microbiology Walkway Era Begins!

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Background: CDC estimates that 48 million (1 in 6 American) get sick from foodborne disease every year, resulting in 128,000 hospitalization and 3,000 deaths. Elderly people are at higher risk foodborne diseases because in geriatric the gastrointestinal tract in geriatric holds food for a longer time allowing the bacteria to grow, in addition to having reduction in the production of acid in the stomach, decrease liver and kidney functions to eliminate bacteria and toxins, and underlying chronic disease. Although symptoms might differ depending on the responsible agents, diarrhea is a common symptom. Diagnosing foodborne illness relies on identifying the causative agent stool culture which takes several days to get the results; the BD MAX™ is the new promise for better sensitivity, specificity and faster turnaround.**Methodology:** The BD MAX™ Enteric Bacterial Panel performed on the BD MAX™ System (Becton Dickinson) is an automated test performed directly on unpreserved soft to diarrheal stool specimens or Cary-Blair preserved stool specimens from symptomatic patients with suspected acute gastroenteritis, enteritis or colitis. It detects, utilizing real-time polymerase chain reaction (PCR): *Salmonella* spp*., Campylobacter* spp*. (jejuni* and *coli), Shigella* spp*. / enteroinvasive E.coli (*EIEC), Shiga toxin 1 (*stx1*) / Shiga toxin 2 (*stx2*) genes (found in Shiga toxin-producing *E. coli* [STEC]) as well as *Shigella dysenteriae*, which can possess a Shiga toxin gene (*stx*) that is identical to the *stx1* gene of STEC. The assay requires 1-2 minute sample prep only. The assay was tested for reproducibility, accuracy, and correlation with the existing methods. A challenge panel comprising 16 positive control strains and 16 negative controls were evaluated over three days to determine the accuracy of the assay by correctly identify bacteria tested. Reproducibility was tested testing 4 positive and 4 negative controls over 4 runs. Correlation was done using 20 stool samples; the results were compared to the results obtained from culture performed on the same samples. Campylobacter results were compared to the antigen assay using Meridian Bioscience Immuno*Card* STAT!® CAMPY. Statistical analysis was done using Analyse-it. **Results:** All strains from the challenge panel were correctly identified or excluded by the BD MAX™. Accuracy was 100% and reproducibility was 100%. All patients tested correlated with the culture giving 100% agreement between BD MAX™ and the stool culture or with the Immuno*Card* STAT!® CAMPY. **Conclusion:** The BD MAX™ gave the benefit of a fully automated, high precision, accuracy and acceptable sensitivity assay. The faster turnaround gave the physician the ability to diagnose foodborne illness within hours instead of relying on the culture which takes several days, and to prescribe the adequate antibiotics if necessary which will help the antibiotic stewardship. The walkway platform for the microbiology laboratory is here, looking forward to complete walkway with random access!

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Frequency of Pathogens Isolated from Cerebrospinal Fluid in Brazilian Public Hospitals

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Background: Bacterial and fungal meningitis represent central nervous system infections that can be community or hospital-acquired. Regardless of the source of infection, the rapid and accurate identification of an infectious agent is an emergency situation and the suspected individuals require fast evaluation and treatment. The purpose of this study was to describe the frequency of the main pathogens found in the cerebrospinal fluid (CSF) samples and determines the antimicrobial resistance profiles among inpatients at Brazilian public hospitals. **Methods:** We analyzed 5451 CSF samples processed by the clinical microbiology section of AFIP Laboratory during November 2018 to November 2019. The samples were submitted to gram staining, antigen detection by latex agglutination test and microbial culture. An aliquot of CSF sample was used to prepare a gram staining, as well as to inoculate on blood, chocolate and MacConkey agar plates. After inoculation, these agar plates were incubated in aerobic atmosphere for 24-48h. The bacteria identification was performed by MALDI-TOF mass spectrometry (Vitek-MS) and the minimal inhibitory concentrations of antibiotics were determined using the Vitek®2 system, according to the manufacture's recommendations. The MIC value was reclassified as susceptible, intermediated and resistant, according to Clinical and Laboratory Standard Institute (CLSI, 2019) clinical breakpoints. Only one positive CSF sample per patient was considered for descriptive analysis. **Results:** Out of 5451 CSF samples processed, 122 (2.2%) samples showed positive culture for the presence of microorganisms. Out of which, 72 (59 %) were Gram-negative Bacilli (GNB), 41 (33.6%) were Gram-positive Cocci (GPC) and 9 (7.4%) were yeasts. The order of prevalence (top ten) of CSF isolates was *Streptococcus pneumoniae* [24/122 (19.7%)], *Klebsiella pneumoniae* [11/122 (9%)], *Pseudomonas aeruginosa, Enterobacter cloacae* complex and *Acinetobacter baumannii* [9/122 (7.4%)] each one, *Cryptococcus neoformans* [7/122 (5.7%)], *Staphylococcus epidermidis* and *Escherichia coli* [6/122 (4.9%)] each, *Neisseria meningitidis* and *Haemophilus influenza* [5/122 (4.1%)] each. Based solely on pathogen identification, 36/122 (29.5%) cases were classified as community-acquired meningitis and 86/122 (70.5%) were classified as hospital-acquired meningitis. 100% of the *Streptococcus pneumoniae* was susceptible to penicillin, six of them showed the following serogroup: 6C (n=3), 12F (n=1), 15B (n=1) and 16F (n=1). Extended spectrum beta-lactamase (ESβL) rates for *E. coli* and *K. pneumoniae* were 66.7% (4/6) and 90.9% (10/11), respectively. The resistance to carbapenems was observed mainly in 44.4% of the *Pseudomonas aeruginosa*, 77.8% of the *Acinetobacter spp.* and 36.4% of the *K. pneumoniae*. Between the GPC the resistance to oxacillin was observed in 50% of the *S. aureus* and 71.4% of the Coagulase Negative *Staphylococcus*. **Conclusions:** Although, *S. pneumoniae* still remains the main cause of community-acquired bacterial meningitis, it is important to highlight the increase of meningitis cases associated to hospital-acquired pathogens. The microbiologic surveillance is critical to guide appropriate antibiotic therapy through the identification of local resistance profiles.

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Profile of Bloodstream Infections caused by Candida Species

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Background: Bloodstream infection caused by *Candida* species is a life-threatening condition associated with high mortality. The incidence of candidemia caused by non-albicans species are increasing worldwide. There are few studies evaluating the profile of candidemia in developing countries. Methods: It was retrospectively evaluated data from positive blood cultures for *Candida spp.* from 33 private hospitals from São Paulo, Brazil, during January-2019 and April-2019. Blood samples were incubated in bottles at BACTEC FX (BD, USA), the positive samples were plated on blood-agar and incubated for 18-24h at 35°C. The colonies were identified using matrix assisted laser desorption ionization time of flight (MALDI-TOF) platform Vitek MS (BioMerieux, France). Results: During the study period, 46,770 blood culture samples were processed, 5,951 of them were positive (12.7%) and 144 of positive blood cultures isolates (2.4%) were identified as *Candida spp.*, from 73 patients in 25 hospitals. Four patients had more than one *Candida* species in blood samples collected in different days. Among 77 candidemia episodes, in 23 (29.9%) *C. albicans* were identified, whereas 54 (70.1%) were caused by non-albicans *Candida* species. The most frequent species in this group were 22 *C. parapsilosis* complex (28.5%), 21 *C. tropicalis* (27.3%) and 5 *C. glabrata* (6.5%). Individuals were older in *C. albicans* group (median: 64, interquartile range: 44-72 years-old) compared with non-albicans group (median: 58.5, interquartile range: 33.5-74.3 years-old) and there was a predominance of male individuals in both groups (albicans: 56.5%, non-albicans: 55.5%). There were four episodes of candidemia in neonates, one (25.0%) caused by *C. albicans*, the other three (75.0%) were identified non-albicans *Candida spp.* In individuals >= 65 years-old, among 31 candidemia episodes, 11 (35.5%) were caused by *C. albicans*, whereas 20 (64.5%) were attributed to non-albicans *Candida spp.* Conclusions: The most common *Candida* species identified in blood cultures in our study was *C. albicans*, however it was observed a high prevalence of candidemia caused by non-albicans species. This high prevalence is also observed in neonates and individuals >= 65 years-old. Non-albicans *Candida spp.* is associated with high prevalence of resistance to azoles and candidemia caused by these agents are a therapeutical challenge. The knowledge of the frequency of these agents as cause of candidemia help to choose ideal empirical antifungal therapy and the identification of the agent is essential for definitive treatment.

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Antimicrobial Susceptibility Profile of Salmonella spp. Isolates in Blood Cultures

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Background: Bloodstream infections (BSI) caused by *Salmonella spp.* are usually secondary to bacteremia caused by gastrointestinal infections caused by these agents. *Salmonella spp.* have limited resistance mechanisms and are usually susceptible to most antimicrobial classes. It is being observed in last years an increase in resistance to fluoroquinolones in *Enterobacterales* worldwide, including *Salmonella spp.* There are few studies evaluating susceptibility profile of *Salmonella spp.* causing BSI. Methods: There were retrospectively evaluated data from positive blood cultures for *Salmonella spp.* from 33 private hospitals from São Paulo, Brazil, during January-2019 and April-2019. Blood samples were incubated in bottles at BACTEC FX (BD, USA), the positive samples were plated on blood-agar and incubated for 18-24h at 35°C. The colonies were identified using matrix assisted laser desorption ionization time of flight (MALDI-TOF) platform Vitek MS (BioMerieux, France). The susceptibility tests were performed using Vitek 2 (Biomerieux, France) and interpreted using Clinical Standards Laboratory Institute (CLSI) criteria. Results: During the study period, 46,770 blood culture samples were processed, 5,951 of them were positive (12.7%) and 23 (0.4%) were identified as *Salmonella spp.*, from 17 individuals, from 12 different hospitals. The median positivity time of these isolates was 27 hours (range: 6-79 hours). There was a predominance of male individuals (64.7%) and median age was 47 years-old (interquartile range: 11.5-66.5 years-old), four patients (23.5%) were under 18 years-old. Considering each episode of bacteremia by *Salmonella spp.*, it was observed that two isolates (11.8%) were resistant and four (23.5%) intermediary for ciprofloxacin, one isolate (5.9%) was resistant for trimethoprim-sulfamethoxazole and all were susceptible for ceftriaxone. Conclusions: We observed a low prevalence of blood cultures positive for *Salmonella spp.*, however almost a quarter of these infections occurred in individuals under 18 years-old. There was a high frequency of *Salmonella spp.* resistant or intermediary for ciprofloxacin, a first-line agent to treat this condition, limiting therapeutic options and influencing in empirical therapy decision.

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Clostridioides difficile Infection in Brazil: Data from a Clinical Parasitology Laboratory

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Background: *Clostridioides difficile* is one of the most important etiological agents involved in antibiotic-associated diarrhea. *Clostridioides difficile* infection (CDI) is a significant nosocomial diseases due to the increase in morbidity, mortality and costs associated with the treatment. The definitive diagnosis for CDI is based on cytotoxicity assays and toxigenic culture regarded as the gold standards methods. However, clinical laboratories are using enzyme-immunoassay, toxin detection kits and molecular biology methods, which are considered faster. The aim of this study was to determine the frequency of *C. difficile* detected by rapid membrane enzyme immunoassay for the simultaneous detection of glutamate dehydrogenase (GDH) antigen and toxins A and B in a single reaction using three specific antibodies. **Methods:** From January to December 2019, the clinical parasitology section of AFIP Laboratory processed 2094 fresh stool samples using the Techlab® C. diff Quik Chek Complete®, for all the samples. To perform the test, 25µL of the stool sample was added to a tube containing 750µL of the diluent and conjugate. This mixture was transferred to the device sample well and incubated at room temperature for 15 minutes. After incubation a wash buffer was added, followed by addition of the substrate to the reaction window. The results were read after 10 minutes. The presence of GDH and/or toxins was indicated by the appearance of a color bar in the appropriate detection zone, according to the manufacturers' recommendations. Only one positive stool sample per patient was considered for descriptive analysis. **Results:** Of the 2094 stool samples, 110 (5.26%) were positive for GDH, with positive screening for the presence of *C. difficile*, and were also confirmed for the presence of toxigenic *C. difficile* strain by detection of toxin A and toxin B, 1968 (94%) were negative for GDH and toxins and 3 (0.14%) samples showed indeterminate result. The prevalence of CDI was 62 (56.4%) and 48 (43.6%) among female and man patients, respectively. Median age of patients with CDI was 56.6 years old (range, 1 - 94 years). The main age range was 50-94 years comprised 67.2% of cases. A total of 70% of *C. difficile* were detected in patients assisted by public health service and 30% assisted by the private health service. We did not have access to patient's symptoms, clinical history or further diagnostic procedures. **Conclusion:** Even with the increase of CDI cases in worldwide, very little is known about the incidence and epidemiology of CDI cases in Brazil. Although, our study had some epidemiological limitations, it is important to share with the scientific community these preliminary data from Brazilian scenario.

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Carbapenem Inactivation Method for Carbapenemase Detection in a Routine Microbiology Laboratory: Comparison with Whole Genomic Sequence and Phenotypical Tests

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Introduction: Infections caused by gram-negative organisms resistant to carbapenems are associated with high mortality, increased hospital length of stay and higher costs. The most important resistance mechanism to carbapenems in *Enterobacterales* is the production of carbapenemase enzymes. Modified carbapenem inactivation method (mCIM) is a phenotypical test used to detect the presence of a carbapenemase and its complimentary test with EDTA (eCIM) detects if the enzyme is a metallo-betalactamase. The identification of the resistance mechanism is important for therapeutic and epidemiological purposes. The objective was to evaluate the performance and applicability of this methodology for carbapenemase detection in a routine microbiology laboratory. Methods: A TOTAL OF 25 ISOLATES of carbapenem-resistant *Enterobacterales* (CRE) were evaluated by mCIM/eCIM. We have selected 19 isolates of CRE from a sample collection from a research laboratory with molecular characterization by whole genome sequencing (WGS), using Illumina Inc. platform (USA). In addition, 6 isolates of CRE from a routine microbiology laboratory were also included, these isolates had only phenotypical characterization for carabapenemase production, using modified Hodge test and EDTA. All isolates had carbapenem resistance screened using Vitek 2 platform (Biomerieux, France) and confirmed by Kirby-Bauer disc diffusion method. There were performed mCIM and eCIM for all selected isolates. The isolate was initially incubated for 4 hours in a liquid culture media with a disc of meropenem, after the disc was plated in a blood-agar containing *Escherichia coli* ATCC 25922 and incubated again for 18-24h at 35°C, and then the inhibition zone was interpreted. Kappa-index was calculated to evaluate agreement. Results: Considering 19 isolates with WGS, there were 15 *Klebsiella pneumoniae* and four *Serratia marcescens*. Among *K. pneumoniae*, 10 were KPC-producer, one NDM-producer and four did not present a carbapenemase gene. There were two KPC-producer *S. marcescens* and two non-carabapenemase producers. All KPC isolates were mCIM positive and eCIM negative, the only NDM isolate was both mCIM and eCIM positive. Among six non-carbapenemase producer isolates, there were three mCIM positive, two *K. pneumoniae* and one *S. marcescens*. Kappa-index comparing mCIM with WGS was 0.578 (agreement: 84.2%) and eCIM with WGS was 1.0. Considering the four phenotypically characterized isolates, there were three *K. pneumoniae*, two of them Hodge positive and onde EDTA positive, and one *E. coli* Hodge negative. Two Hodge positive isolates were also mCIM positive, one EDTA positive was eCIM positive, and both Hodge negative isolates were mCIM negative. Kappa-indexes comparing mCIM with Hodge and eCIM with EDTA were both 1.0. Conclusions: The modified carbapenem inactivation method is an easy, cheap and affordable method for carbapenemase detection to be implemented in a routine microbiology laboratory. However, we observed a moderate agreement comparing mCIM with WGS, and we observed an almost perfect agreement comparing mCIM with Hodge and eCIM with WGS and EDTA.

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Prevalence of Resistance to Ceftolozane-Tazobactam in Pseudomonas aeruginosa in Brazil

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Background: Ceftolozane-tazobactam (CFT-TZB) is a second generation betalactam-betalactamase inhibitor used for treatment of infections caused by multidrug resistant gram-negative organisms, specially *Pseudomonas aeruginosa*. The most important

resistance mechanism for this antimicrobial agent is the production of carbapenemases. There are few data about the prevalence of resistance to CFT-TZB in developing countries. Methods: It was retrospectively evaluated data about positive cultures from 33 private hospitals in the city of São Paulo, Brazil, during 01-20-20 and 02-05-20. Blood samples were incubated in bottles at BACTEC FX (BD, USA). Positive blood cultures and other clinical samples were plated on blood-agar and incubated for 18-24h at 35°C. The colonies were identified using matrix assisted laser desorption ionization time of flight (MALDI-TOF) platform Vitek MS (BioMerieux, France). Susceptibility tests were performed using Vitek 2 (BioMerieux, France). Minimum inhibitory concentration (MIC) for CFT-TZB were determined using gradient diffusion method MIC test strip (Liofilchem, Italy) for all carbapenem-resistant *Pseudomonas aeruginosa* (CRP) isolates. It was used Clinical Standards Laboratory Institute (CLSI) interpretative criteria for antimicrobial susceptibility tests. Results: There were 12,199 cultures from clinical samples, during the study period. We have observed among 32 CRP isolates, four (12.5%) resistant to CFT-TZB, in 15 hospitals. The MIC_{50} for CFT-TZB was 1.0 mg/L and MIC_{90} was 4.0 mg/L. There were 20 CRP isolates from 107 positive respiratory samples (10.3%), two (10.0%) were resistant to CFT-TZB. Among 330 positive tissue fragments and other secretions, there were six CRP (0.9%), one (16.7%), from a bone fragment, resistant to CFT-TZB. Considering 1,696 positive urine cultures there were isolated 3 CRP (0.04%), one of them (33.3%) CFT-TZB-resistant. There were none CFT-TZB-resistant among three (0.06%) CRP isolated from 732 positive blood cultures. Conclusions: CRP were more frequently isolated from respiratory samples. Despite of the fact, CFT-TZB is considered an optimal antimicrobial agent against *P. aeruginosa*, we have observed a high prevalence of resistance for this antibiotic. There are limited resources to evaluate the carbapenem resistance mechanism for *P. aeruginosa*, challenging the prediction of the mechanism for CFT-TZB use.

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Rapidly Assessing the Host Immune Response for the Diagnosis of Sepsis, a Prospective Multi-Site Clinical Study in the Emergency Department (ED) Employing the Leukocyte Structural Index (LSI)

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Introduction: Early recognition of sepsis is integral in the initiation of aggressive therapy targeted at reversing physiologic derangements which, if left untreated, often lead adverse outcomes, including death [1]. ED clinicians must balance the benefits of early intervention against the risks of indiscriminate use of resource-intensive interventions. Currently, no rapid diagnostics exist with clinically actionable performance for early sepsis diagnosis in acute care environments. We previously demonstrated the performance of the LSI, an assay that utilizes deformability cytometry to rapidly quantify the immune activation signatures of sepsis, in a high acuity cohort of adults presenting to the ED with signs of infection and organ dysfunction [2]. Here we present the diagnostic value of the assay in a multi-site, out of sample prospective cohort of adults presenting to the ED with signs or suspicion of infection. **Methods:** The LSI assay uses a benchtop instrument designed for the clinical laboratory with blood-to-answer turnaround time <10 minutes. The sample preparation, analytical testing, and reporting are fully automated with two operator interaction points (initial aliquot of 100uL and intermediate sample transfer step; total hands-on time <90 seconds). The assay's reporting range is divided into three interpretation bands corresponding to regions of low, intermediate, and high probability for sepsis. Test results in the intermediate band (test likelihood ratio ~1) are considered diagnostically indeterminate. Adult patients presenting to the ED with signs or suspicion of infection (2+ SIRS: one must be temperature or WBC, or order for cultures) were prospectively enrolled at multiple sites in the USA. EDTA-anticoagulated blood was assayed within 3 hours of draw. Subjects were retrospectively adjudicated for sepsis (Sepsis-3 definition) by an independent physician committee. Diagnostic performance characteristics and receiver operating curves were used to examine the assay results (reported as mean (95% CI). Trends in assay score with severity of illness metrics and hospital-based outcomes were also assessed. **Results:** Of the 294 patients enrolled (median age 55 (IQR 40 - 71), in-hospital mortality of 2.4%), 18.0% were adjudicated as septic. The assay demonstrated 0.87 (0.69 -0.95) AUC, 85% (65 - 93) sensitivity, 85% (65 - 93) specificity, 97% (80 - 99) NPV, and DOR of 31.7 (15.1 - 47.5), with the interpretation bands' two cutoffs. 27% of subjects were in the intermediate band. Additionally, SOFA ($p < 0.001$), PIRO ($p < 0.001$), APACHE II ($p < 0.01$) and in-hospital mortality rates ($p < 0.05$) were significantly lower, while hospital-free days ($p < 0.001$) were significantly higher for subjects in the low versus high probability of sepsis bands. **Discussion:** The LSI assay achieves clinically-actionable diagnostic performance, risk-stratifying patients with the highest likelihood of developing sepsis in an ED population. Furthermore, the assay is designed for the acute care setting utilizing routine blood draw samples, requiring minimal operator input, and achieving a blood-to-answer turnaround time of <10 minutes. It is anticipated that the assay will improve the identification, management and treatment of sepsis.

1.Friedman, K., et al., J Crit Care Med, 1998.2.Sheybani, R., et al., Crit Care *ISICEM*, 2019.

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Atypical Infection after Silicone Implant in Breast Implants: A Rare Case Report Involving the Genus Nocardia spp.

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Background: Nocardiosis is a severe, opportunistic inflammatory infection that usually affects immunocompromised patients. The most common form of the disease refers to chronic lung infection after inhalation of aerosols from the environment and, in less frequent occurrence, may occur through trauma-induced local abscesses. **Objective:** To describe a rare case report of infection by the genus *Nocardia* spp after silicone implant in mammary prostheses. **Case Report:** The 46-year-old female patient had an intense and atypical infection, encapsulated in the right breast, after a surgical procedure to implant silicone. **Methods and Results:** Tissue excision was performed and the material was collected for microbiological and genetic diagnosis. After the incubation period, the growth of rough and dry colony was started in Lowestein-Jensen medium, partially acid resistant alcohol was present through the modified ZiehlNeelsen staining, with filamentous and Gram-branched bacilli. The microorganism was submitted to three protocols for identification by mass spectrometry MALDI-TOF MS, but without success, probably due to the scarcity of data from this microorganism in the Software database. Regarding the genetic diagnosis, the 16S ribosomal gene was sequenced by Sanger to identify the bacterial genus. From the comparison of the sequence obtained with the NCBI-BLAST database, the existence of bacteria of the genus *Nocardia* spp was confirmed.

Conclusion: This case report serves as an alert to health professionals for a type of atypical infection in secretion samples after breast implants and for future studies on improvements in the techniques of commercial identification, cultivation and susceptibility testing for *Nocardia* spp.

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Clinical Validation for Group B Streptococcus (GBS) Screening using Cepheid Xpert GBS Assay in Vaginal/Rectal Fluids

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Background: Group B *Streptococcus* (GBS) are opportunistic bacteria also known as *Streptococcus agalactiae.* GBS can cause clinical infection in pregnant women, being mainly of asymptomatic colonization. Studies show that women with prenatal colonization have a 25 times greater predisposition to give birth to children with early GBS disease. Thus, the screening of these bacteria is indicated for all pregnant women between 35 and 37 weeks of gestation. GBS routine screening is usually performed by culture-based methods, but such techniques are laborious and the results can take three to five days to be released. **Objective:** Validate the Cepheid Xpert GBS Assay (Sunnyvale, CA, USA) for GBS detection in vaginal and rectal swab samples by Real-Time PCR (qPCR). **Methods:** Two paired vaginal/rectal swabs (Regular Rayon Swab with Stuart Agar Gel; COPAN) from 40 pregnant women (20 positive and 20 negative) were selected for this validation. The results were previously submitted for routine prenatal GBS screenings on broth-enriched cultures (Group B *Streptococcus* Selective Culture test). Xpert GBS tests (Cepheid) were performed according to manufacturer's instructions. **Results** Culture-Based and qPCR-Based methods were 100% concordant. Both clinical specimens (vaginal/rectal swabs) presented similar performance. The Cepheid Xpert assay demonstrated a great reproducibility of the results in comparison with the culture assay. **Conclusion:** GBS screening in pregnant women is important for intrapartum antibiotic prophylaxis, preventing vertically acquired GBS infection. Both culture-based (gold-standard) and qPCR-based techniques are useful for GBS detection, without loss of sensitivity and specificity. However, Cepheid Xpert assay was shown to be an excellent alternative since it is able to release results in less than sixty minutes, enabling greater agility in making clinical decisions. The integration of sample preparation and PCR in a single automated workflow becomes a test with ease of execution, with results available quickly, being possible to use it

as a potential molecular point of care test. The Xpert GBS test is an automatized amplification system that can play an important role in the rapid and sensitive diagnosis of GBS infections.

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Malaria, What Happened in Brazil in Last Years?

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Background: Malaria has had a greater impact on world history than any other infectious disease. According to WHO estimates, the global tally of malaria in 2018 was 228 million malaria cases and 405,000 malaria deaths worldwide. In Brazil was registrated 151,966 cases in Brazilian Amazon Region, and 354 cases in Extra-Amazon region. In areas outside the Amazon, more than 80% of registered cases are imported from states belonging to the endemic area. **Objective**: To describe the epidemiological characteristics of malaria infection in Brazil during the period of January 2008 to January 2020. **Methods**: This was a retrospective study, carried out through consultation of laboratory test (Immunochromatography and ELISA) results from Hermes Pardini Institute web LIS, during period 2008 to January 2020. Epidemiological data such as gender, age and region were statistically analyzed. **Results:** A total of 8,200 patients from all over the country were evaluated, and 73.6% were male and 26.4% were female. The rate of positivity for Malaria was 36% for the whole period evaluated. Of this total, 72% were male and 28% were female, and the prevalence/year was distinct (Figure 1). About age group, the distribution was 10.7% up to twenty years, 21.5% between 21 to 30 years, 24.8% 31 to 40 years, 18.9% 41 to 50 years, 13.1% 51 to 60 years, 7.4% 61 to 70 years and 4.1% were over 70 years old. There was a predominance of patients from the Southeast region (51.1%) followed by the Midwest (15.3%), Northeast (15.1%), North (9.1%), and South (9.4%). **Conclusions**: The distribution of malaria is heterogeneous in Brazil and the disease is present in all regions of the country. Even with the adoption of the global integrated malaria control strategy, it continues to be a challenge and should be associated with government programs for economic development in all regions.

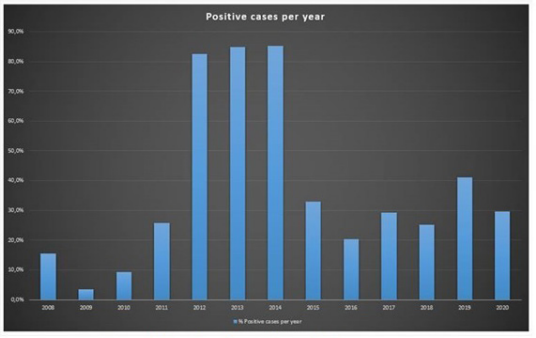

Figure 1: Number of malaria positive cases.

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Landscape of More Than Decade of Syphilis in Brazil - 2008 to 2019

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Background: Syphilis is a genital ulcerative disease caused by *Treponema pallidum pallidum*. Transmission occurs through sexual contact, and it is associated with significant complications. The World Health Organization estimates that 12 million new cases of syphilis occur each year. An increase in incidence of disease has been reported, especially in developing countries which emphasizes the need for a more accurate epidemiological survey. **Objective**: To describe the epidemiological characteristics of syphilis infection in Brazil (according Venereal Disease Research Laboratory test-VDRL) during the last twelve years. **Methods**: This study was a retrospective study, carried out through consultation of VDRL test results obtained from patients who were tested for anti-*Treponema pallidum* IgM antibody in Hermes Pardini Institute care routine. The data were obtained in period of January 2008 to December 2019. Epidemiological data such as gender, age and region of patients were statistically analyzed. **Results:** A total of 1,047,201 patients from all over the country were evaluated between 2008 and 2019. There was a predominance of patients from the Southeast region (82.8%), followed by the Midwest (7.8%), Northeast (6.7%), South (2.1%), and North (0.5%). The rate of positivity for VDRL was 6.49% for the whole period evaluated. Of this total, 50.9% were male and 49.1% were female, and the prevalence per year is shown in Table 1. About age group, the distribution was 2.7% up to fifteen years, 9.5% between 15 to 20 years, 34.0% 21 to 30 years, 24.0% 31 to 40 years, 13.3% 41 to 50 years, 8.8% 51 to 60 years, 4.3% 61 to 70 years and 3.4% were over 70 years old. **Conclusion**: The results obtained showed an increase number of syphilis positive cases in the last twelve years. The high prevalence rate observed can be associated with sexual rick behavior and/or recreational drug use.

Table 1 – Prevalence of VDRL positive cases per year

2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
3.32%	3.21%	3.56%	4.23%	6.00%	5.37%	5.95%	6.30%	7.53%	6.37%	8.34%	7.72%

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Hip Joint Infection by Propionibacterium avidum: Etiology Defined by Automated Diagnostic Techniques

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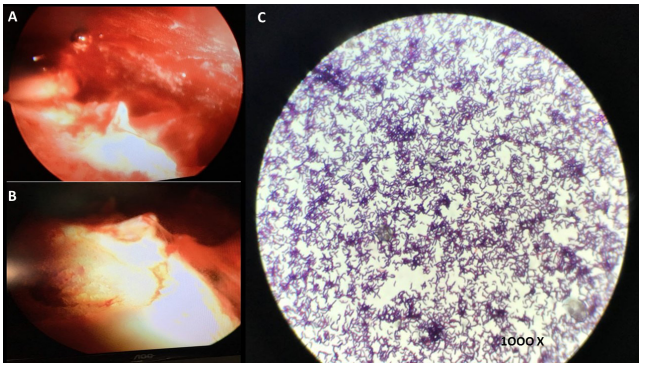
Background *Propionibacterium* spp. are representatives of human skin microbiota. They are Gram-positive, nonmotile, nonsporeforming, anaerobic bacilli. In contexts as obesity, arthroplasty, immunosuppression and trauma they can be pathogens, causing superficial or deep/invasive infections. Joint infection has been described, although *Propionibacterium avidum* is implicated in a few cases. ***Case report*** Male, 29 years old, bodybuilder and regular user of anabolic steroids, suffered a car accident, with trauma to left hip, causing fracture of femoral neck. He underwent osteosynthesis with a pin plate. After 8 days, patient began to feel pain, with no signs of wound infection. Anti-rotating cannula has been added to improve stability. Patient persisted with pain and had elevated CRP and ESR. Radiologic examinations showed decreased joint space and massive effusion. Hip arthroscopy revealed joint destruction and synovitis (Figure 1A/B).Three samples of synovial fluid were collected in aerobic blood cultures BD bottles (Becton-Dickinson, USA). Flasks were incubated on BD BACTEC FX ™ (Becton-Dickinson, USA) and, after about 72h, were detected positive. Samples were sown on 5% sheep blood agar (PlatLabor, Brazil) and chocolate agar (BioMérieux, France). After 48h at 37°C incubation, convex and white colonies were observed. Gram stain revealed non-sporulated Gram positive bacilli (Figure 1C). *Propionibacterium avidum* was identified by MALDI-TOF on Vitek MS (BioMérieux, France). Total hip arthroplasty was performed. Intravenous antibiotics was maintained for 4 weeks, followed by hospital discharge with oral antibiotics for 6 weeks. Patient remains stable at 22 months after surgery, maintaining bodybuilding activities and using anabolic steroids. ***Conclusion*** *P. avidum* is commensal in cutaneous microbiota. Identification of this microorganism can be difficult because it is confused with a contaminant. In addition, its slow growth can be overlooked if cultures are discarded after 3 days. In this case, incubation in blood culture bottles and identification by MALDI-TOF contributed to diagnosis and clinical management.



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Biotin Pull-Down and Mass Spectroscopy Evaluation of Essential Proteins of a Neglected Tropical Disease: Human African Trypanosomiasis

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Background: Neglected tropical diseases are a distinct group of tropical infections that occur commonly in economically depressed regions. Human African Trypano-

somiasis (HAT) a parasite, known as African sleeping sickness, is prevalent in the Sahara region of Africa. A recent survey in 2018 estimated 1,000 people suffered from this disease sometimes resulting in death. The objective of this current research is to pull-down proteins of interest that are essential for HAT. The advantage of this assay is the customizability of the biotinylated streptavidin complex.

Methods: Pull-down assays are prevalent *in vitro* procedures for the identification of proteins associated with an immobilized biotin-streptavidin small molecule. The small molecule chosen in this study is a drug that is used *in vitro* to treat the HAT infection causing the parasite to die within 48 hours at low micromolar concentrations. The protein(s) that the drug interacts with to effect treatment has not been identified, which presumably would be identified by the assay. This method follows a three-step procedure starting with synthetic creation of the biotinylated small molecule. The synthetic procedure follows a simple one-step reaction in mild conditions with purification by recrystallization. Characterization of the structure was done by carbon and hydrogen NMR followed by LC-MS. The assay uses a parasite cell line (lister 427) at an amount of 1 X 10^7 per milliliter. The cells are sonicated for 10 cycles at 5 seconds a cycle with a 30-second cycle break at 0° C. After disruption of the membrane by sonication the lysate is then incubated with the biotinylated compound for one hour to allow for optimally time for binding interactions. Followed with the introduction of streptavidin beads as the stationary phase to allow for a non-covalent interaction between the biotinylated targets and the beads. Making the selection of biotinylated proteins selective over other non-specific proteins interactions. A series of washes take place to remove any non-specific binding and is followed with the introduction of pure biotin to competitively knock off the biotinylated ligand-protein complex. The proteins were reduced and alkylated with DTT and iodoacetamide then underwent in-solution trypsin digestion. The sample was then run on an Orbitrap Elite mass spectrometer system. A Uniport database search of the mass spectroscopy report gave 7 parasite-specific proteins of interest.

Results: Proteins were identified by in-solution trypsin digestion and mass spectrometric identification of peptide sequences using an orbitrap trap mass spectrometer. They are, Flagellar attachment zone protein 1, IgE dependent histamine-releasing factor, Kinetoplastid membrane protein, Microtubule-associated protein, Peptidyl propyl cis-trans isomerase, Thioredoxin, and Universal minicircle sequence binding protein. Two independent repeats were done for validation

Conclusion: A pull-down assay is a valid method to understand interactions at the molecular level allowing for targets of interest to be identified. Pairing the method with mass spectroscopy allows for the detection of proteins and in this study, we have identified 7 parasite-specific proteins that are under further investigation for the molecular mechanism of action. These proteins are potential plasma biomarkers for diagnosing a HAT infection.

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Development of HBcrAg quantitative assay for fully automated analyzer LUMIPULSE L2400 with automated pretreatment process

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Background: Hepatitis B virus core-related antigen (HBcrAg) consist of three HBV antigens, HBeAg, HBcAg and HBV precore protein p22cr, and its levels reflect serum HBV DNA levels and intrahepatic cccDNA levels. Present HBcrAg quantitative reagent (Lumipulse G HBcrAg) has issues to be addressed; manual pretreatment process and sensitivity. To address these issues, we developed the next generation HBcrAg assay prototype on fully automated chemiluminescence enzyme immunoassay (CLEIA) system, Lumipulse L2400 analyzer, using iTACT (Immunoassay for Total Antigen including Complex via pre-Treatment) protocol. We evaluated fundamental performance of the prototype assay, iTACT-HBcrAg.

Methods: This novel HBcrAg assay is a two-step sandwich CLEIA with the on-board ITACT pretreatment. All evaluations were executed on LUMIPULSE® L2400 analyzer (FUJIREBIO INC.). The running time of the prototype assay is 32.5 min including 6.5 min pretreatment process. It requires 35 µL of serum or EDTA-2K plasma. Within-run precision, matched pair correlation between serum and plasma, dilution linearity, spike recovery using HBcAb negative/positive and HBeAb negative/positive and negative distribution were evaluated. The correlation with the present conventional assay, Lumipulse G HBcrAg (cv-HBcrAg) (FUJIREBIO INC.), was assessed. Size exclusion chromatography (Superdex200 Increase 10/300 GL, GE) was executed by using an HBcrAg positive specimen for molecular analysis of HBcrAg.

Results: Within-run precision (% CVs) for the novel assay ranged from 1 to 3% when 3 different concentration of control samples. LoQ was less than 2.3 LogU/mL. Correlations between serum and plasma using matched pair samples, and between HBcrAg levels by the novel assay and by cv-HBcrAg for HBsAg positive samples over 3.0 LogU/mL were well. Dilution linearity and spike recovery satisfied our criteria (100% ±10%) regardless presence of HBcAb and HBeAb. The measurement value in HBV DNA negative specimens (n=215) was distributed from 0 LogU/mL to 2.0 LogU/mL. The effects of pretreatment of samples were evaluated with size exclusion chromatography. Immuno-reactive materials in pretreated specimens were detected in fractions corresponding to the molecular mass of monomer size in contrast to those corresponding to higher molecular mass from the non-treated specimens, indicating that HBcrAg molecules were extracted from different molecular structures as virions, empty particles and dimeric forms of HBeAg by pretreatment.

Conclusion:The novel HBcrAg assay using iTACT protocol showed higher sensitivity than cv-HBcrAg and reduced turn around time (TAT) by the contribution of on-board pretreatment process. These features especially improved sensitivity would provide novel clinical utilities in treatment for HBV infected patients.

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Eliminate the Cold-Chain Transport of Samples for Bacterial Testing: A Clinical Study

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Background: Clinical microbiology laboratories are increasingly centralized to help contain costs, increasing the need for costly rapid refrigerated transport from the patient to the laboratory. During these transports, breaks in the cold-chain occur frequently and adversely affect the sample, especially if the number of organisms is low ($\leq 10^1$ cfu). We developed a transport system (LabReady®), that eliminates the need for costly rapid cold-chain transport of samples. In a clinical study, we compared LabReady against the current standard using group B streptococcus (GBS).

Methods: Vaginal-anorectal swab samples were obtained by healthcare providers with both the LabReady GBS system and BD BBL Culture Swab™ Plus system. Clinical sites were located in New Jersey, Colorado, Louisiana, or Utah. Subjects were randomized to either the LabReady or BBL system sample collected first. The LabReady system was transported in ambient temperature and the BBL system under refrigerated conditions (per manufacturer), to the central laboratory in New York in <24 hrs, as recommended by the CDC. LabReady sample was enriched during transport while the BBL sample was enriched immediately upon arrival at the laboratory. The centralized reference laboratory performed both: 1) GBS Culture and Remel™ PathoDX™ Strep Grouping, and 2) BD MAX™ GBS Assay (PCR) on both samples immediately following enrichment. Standard statistical methods were used. Inclusion criteria were female ≥ 15 years of age, pregnant at 35 weeks and 0 days to 37 weeks and 6 days gestational age, written informed consent/assent, able and willing to provide two vaginal-anorectal swab samples. Exclusion criteria included no fetal heart activity detected, placenta previa, active labor, antibiotics within the past 14 days and previously participated in this study. All institutional IRBs and a central IRB approved of the study.

Results: Of 300 projected patients, we report results from 76 completed patients. Mean demographics observed were age 31 years old, gestation 36 weeks and 1 day and 20% were Black. Despite strict control, 4 paired samples did not arrive until 48 hours after collection. All samples were included for analysis and results. With BD MAX GBS (PCR) testing, one BBL sample was negative while the paired LabReady sample was positive for GBS. Results for PCR testing were: prevalence 22%, sensitivity 100% (95% CI:82%-100%), specificity 98% (95%CI:91%-100%), accuracy 99% (95%CI:93%-100%), and ratio of positive results was 1.05. With culture and strep group testing, there were 3 inconsistent results comparing the LabReady to BBL systems. Results for culture testing were: prevalence 23%, sensitivity 76% (95% CI:53%-90%), specificity 98% (95%CI:91%-100%), accuracy 93% (95%CI:85%-97%), and ratio of positive results was 0.94. The LabReady system saved the laboratory an average of 23 hours and 02 minutes for reporting PCR or culture results.

Conclusion: The transport of GBS samples via either ambient temperature (LabReady system) or refrigerated temperature (BBL system) did not result in significantly different testing results whether using PCR or culture to test the samples. LabReady appears to be a much less expensive, simpler and faster alternative to cold-chain transport of samples from the patient to the laboratory.

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Evaluation of Syphilis Incidence in a Brazilian HIV Positive Cohort

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Background: : According to the World Health Organization (WHO), sexually transmitted infections (STIs) are considered a major public health problem, and these infections are common everywhere in developed and developing countries. The co-infection of STIs is usually between Human Immunodeficiency Virus (HIV) and syphilis, gonorrhea or Herpes. According to the CDC, around half of men who have sex with men (MSM) diagnosed with syphilis were also HIV positive in the US. In Brazil, HIV and syphilis prevalence are 26 and 75.8 cases/100.000 habitants, respectively (2018). Efforts are being made to develop a series of cross-sectional studies to monitor risk behavior practices of most-at-risk populations for STIs, especially HIV and syphilis infection, at the national level. **Objective:** To evaluate syphilis incidence in patients who tested viral burden of HIV on Hermes Pardini Institute, in Brazil. **Methods:** This was a retrospective study, carried out through consultation of laboratory test results from Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) web LIS. Random sampling results of viral burden of HIV, obtained from January 2018 to January 2020 were compiled (n=219). Patients who presented detectable HIV-1 pro-viral DNA by in-house PCR assay were included in the HIV positive group (n=90), whereas undetectable HIV-1 pro-viral DNA in the HIV negative group (n=129). A total of 147 (n = 70 HIV+/77 HIV-) patients presented results for Treponemal IgM (Alinity Syphilis TP - Abbott) and/or IgG (FTA-Abs Sifilis - WAMA Diagnóstica) and 91 (n= 30 HIV+/61 HIV-) for VDRL screening (RPR BRÁS - Laborclin). Two-sample t-test and Chi-squared independence test were applied for statistical analysis and $p< 0.05$ was considered as a significant result. **Results:** The HIV positive group showed more patients with reactive Treponemal IgM and/or IgG serology and reactive VDRL rates (74.29%, 53.33%, respectively) compared to the HIV negative group (18.18%, 9.84%, respectively) ($p<0,001$). Syphilis incidence in the HIV positive group showed to be higher in patients between 31-40 years old (35.29%) and from the Southeast region of the Brazil (52.94%). On the other hand, in the HIV negative group, incidences in age group was higher in the 21-30 years old and in the Midwest region (50%). HIV infection has demonstrated strongly association with Treponemal antibody ($X^2= 46.65$ $p=0$) and VDRL ($X^2= 20.75$ $p=0.000005$) reactivity. **Conclusion:** The number of HIV positive patients who have been infected by syphilis in their lifetime is substantially larger than in the HIV negative group. This fact could be explained by an associated risky sexual lifestyle. Moreover, it is known that HIV positive patients are more susceptible to co-infection, including syphilis, than healthy patients. In this context, a there is a need to expand access to public health services, providing prevention and to avert HIV infection and other STIs.

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Aptamers that Bind Filovirus GP: A New Frontier for Detection and Neutralization of Ebola and Marburg Virus

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Background: Filoviruses, including Ebola virus (EBOV) and Marburg virus (MARV), cause fatal hemorrhagic fever in humans, non-human primates, bats, and other mammals. Filovirus glycoproteins (GP) have proven to be effective therapeutic targets for neutralizing antibodies (nAbs) and vaccine development. However, Abs (50-150kDa) are unable to access crucial epitopes required for virus entry due to GP architecture and the virus entry mechanism. Aptamers are small, structured nucleic acid ligands generated through *in-vitro* selection. When generated against recombinant proteins, aptamers may not interact with the protein in its native context due to conformational differences. Filoviruses display a trimeric GP that undergoes structural transitions (pre-fusion & post-fusion) during entry.

Methods: Here, we developed a platform (SELEX) that allowed us to rapidly generate aptamers that recognize native filovirus GP as displayed on mammalian cells and an engineered, replication-competent vesicular stomatitis virus (VSV). We performed 15 rounds of selection using purified rVSV-MARV-GP viral particles and generated enriched populations specific for rVSV-MARV-GP. **Results:** We identified a panel of aptamers that confirmed the observed binding phenotype (affinity & specificity) of the enriched populations. **Conclusion:** The selection platform used in this study could be extended to identify aptamers for surface proteins of other emerging viruses to accelerate the development of diagnostics, therapeutics, and surveillance technologies.

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The Identification of Mycobacterium Species by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry - MALDI-TOF MS: Different Inactivation and Protein Extraction Protocols

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Background: Some species of the *Mycobacterium tuberculosis* complex (MTBC), particularly *Mycobacterium tuberculosis*, which causes human tuberculosis (TB), are the first cause of death linked to a single pathogen worldwide. The non-tuberculosis (NTM) mycobacteria have potentially pathogenic and opportunistic species, routinely isolated from clinical specimens, mainly from immunocompromised patients. The identification of species of mycobacteria is important to reveal differences in clinical relevance, help in choosing the appropriate therapy and minimize the selection of resistant microorganisms. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) has been used in routine clinical microbiology laboratories to identify species of mycobacteria. Due to the pathogenicity of mycobacteria and the thick cell wall with mycolic acids, inactivation and protein extraction is required. The aim was to evaluate the performance of different inactivation and protein extraction protocols, and MALDI -TOF MS (Vitek MS, Biomerieux) for the identification of mycobacteria from Löwentein Jensen (LJ) solid cultures obtained at the Microbiology Laboratory at Hermes Pardini Institute. **Methods**: A total of 122 strains of mycobacteria (06 ATCC's and 116 previously identified by PRA-hsp65) were included in the study. The isolates were subcultured in both Löwenstein-Jensen medium (LJ) as solid media and the identification by MALDI-TOF MS. The protocols were i) Protocol 1: according to manufacturer's instructions of MALDI-TOF; ii) Protocol 2: centrifugation, heat and agitation of mycobacterial biomass; iii) Protocol 3: inactivation and extraction with 70% ethanol and centrifugation. **Results**: There was disagreement in the identification of 8 strains whose sequencing analysis were compatible with those obtained by MALDI-TOF MS. A total of 5 strains were not identified, of which 03 are not deposited in the Vitek MS database. Protocol 1 had an identification rate of 90.2% (110/122), protocol 2 89.3% (109/122) and protocol 3 94.3% (115/122). **Conclusion**: The results demonstrated the efficiency of the three protocols for mycobacterial identifications, however, protocol 3 was more adequate for the implementation in the laboratory routine and use as diagnostic test, since it presented a higher identification rate and reduced identification turnaround time. The MALDI-TOF MS is a potential tool identify mycobacteria in the clinical laboratory which is important for early initiation of TB therapy, stewardship, infection control/prevention.

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Serological Method Comparison in Chronic Phase Chagas Disease Diagnostics

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Background: Chagas disease represents an important public health issue in Brazil and different regional distributions. The infection occurs mainly in Latin American due to a variety of approximately 140 vector species (*e.g. Triatominae, Hemiptera, Reduviidae*). During chronic phase, the diagnosis is essentially by serology, and medical literature recommends evaluation with an elevated sensitivity test followed by high specificity test. This study presented a comparative method analysis between different serology methods of Chagas disease in Florianopolis region (a low prevalence region), Brazil from the last 10 years.

Methods: Chagas disease IgG serology were analyzed in blood samples between 2010 and 2019, in 4 different methods: hemagglutinatination (HA), chemiluminescence (CLIA), enzyme immunoassay (EIA) and indirect immunofluorescence (IIF) correlating their results with the method and classified as reagent, non-reagent and inconclusive, using a database of a laboratory of the region of Florianopolis, Brazil. Each one of the samples were analyzed by 2 different methods depending on the laboratory routine at the time of collection. As there is no gold standard for Chagas disease diagnostic, specificity and sensitivity tests were performed considering as false results all discordant negative results for sensitivity and all discordant positive results for specificity.

Results: A total of 12,078 determinations were performed for Chagas disease serology. Total results in percentage of reagent samples among the years of 2010, 2011,

2012, 2013, 2014, 2015, 2016, 2017, 2018 and 2019, were 859 (0.46%), 849 (4.94%), 1,719 (5.23%), 2,224 (3.23%), 1,370 (1.53%), 789 (1.39%), 956 (1.46%), 886 (1.91%), 1,194 (1.34%) and 1,444 (3.46%), respectively. The most frequently used method was HA (n=5,129). The combination of methods most frequently used were HA+EIA (n=2,073), HA + CLIA (n=1,632) and HA + IIF (n=1,323). Inconclusive results were 22 determinations (17 EIA and 5 CLIA). The most sensitive methodology during this period was EIA (98,6%) followed by CLIA (96,5%). In terms of specificity, HA had better performance (99,9%) followed by CLIA (99,7%).

Conclusion: Our results demonstrated that HA and CLIA had the best agreement. EIA had the highest sensitivity, however it might result in false positive results, mainly in low prevalence areas. Besides the Brazilian Ministry of Health does not recommend CLIA according to the last Brazilian Guidelines in 2015, CLIA method showed higher sensitivity than IIF and HA, and similar specificity compared to HA.

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Comparative Performance of VITEK 2 and MALDI-TOF MS in Microbial Identification

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Background Since some years, new tools have improved microbial identification. MALDI-TOF MS has been applied in microbiology laboratories aiming fast and precise identification. This study aimed at evaluate performance of Vitek MS (BioMérieux, France) for identification of microbial strains, in comparison with Vitek 2 (BioMérieux, France). **Methods** Clinical samples were seeded in culture media appropriate to each infection site and, after 24 hours of incubation at 37 °C, colonies were submitted to the two methodologies. For Vitek 2 (BioMérieux, France), GN, GP, YST cards were used in case of Gram negative, Gram positive and yeast, respectively. According to manufacturer's guidance, a McFarland 0.5 scale was used for bacteria and McFarland 2 for yeasts. Acceptable result was the identification of microorganisms as a confidence level above 95%. Standard strains ATCC25922 *E. coli*, ATCC27853 *P. aeruginosa*, ATCC25923 *S. aureus* and ATCC14053 *C. albicans* were used as controls. Identification by MALDI-TOF was performed on Vitek MS (BioMérieux, France), using 1 µl of α-cyano-4-hydroxycinnamic acid for bacteria and, in addition to organic matrix, 0.5 µl of 70% formic acid for yeasts. Acceptance result was the identification of microorganism with a percentage of 99,9%, using quality control with ATCC 8739 E. coli strain. Results A total of 139 microbial isolates were tested, including 120 isolates from clinical samples and 19 ATCC strains. Clinical isolates included 30 Gram positive, 30 Enterobacterales representatives, 30 non-fermenting Gram negative bacilli and 30 yeasts, as shown in Table 1. There was absolute agreement between tested methodologies. **Conclusion** Vitek 2 and Vitek MS showed high accuracy in identification of tested microorganisms. However, Vitek MS presents a great advantage, significantly reducing time for etiological definition. This tool represents an important advance for diagnosis in Clinical Microbiology laboratories, favoring a quick and accurate diagnosis, contributing to more assertive and targeted clinical conduct.

Table 1 – Distribution of microorganisms

Microorganism	Number of isolates
Klebsiella pneumoniae	13
Escherichia coli	11
Proteus mirabilis	2
Klebsiella aerogenes	2
Morganella morganii	1
Serratia marcescens	1
Streptococcus agalactiae	15
Enterococcus faecalis	7
Staphylococcus aureus	3
Staphylococcus saprophyticus	2
Staphylococcus epidermidis	2
Staphylococcus capitis	1
Pseudomonas aeruginosa	20
Acinetobacter baumannii	9
Acinetobacter junii	1
Candida albicans	23
Candida tropicalis	4
Candida glabrata	3
Total	**120**

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Implementation of a Multiplexed Screening Assay for Detection of Total Antibodies (IgM/IgG) to *Borrelia burgdorferi*

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Background: Lyme borreliosis is endemic to the state of Maine and causes significant morbidity in the region. Diagnosis of Lyme borreliosis is plagued by issues including: limited opportunities for direct pathogen detection, specificity problems with antibody-based tests, and a generalized lack of sensitivity during early infection when treatment outcomes are most favorable. Currently, the CDC recommends a two-tiered serological approach to overcome some of these issues. Our Maine based reference laboratory uses the standard two-tiered approach and screens over 10,000 samples for antibodies to *Borrelia burgdorferi* with almost 2000 confirmatory tests performed via Western blot each year. In 2019 we evaluated and implemented the BioPlex 2200 Lyme Total (Bio-Rad, Hercules, CA), a multiplexed screening assay for detection of IgM and IgG antibodies directed against *Borrelia burgdorferi* antigens OppA2 (p58), OspC serotype B (OspCB), and a synthetic peptide containing optimized sequences from both FlaB and a modified VlsE.

Methods: During the evaluation period 185 samples were tested retrospectively, and 279 samples were prospectively tested. All samples had been submitted to the laboratory for routine Lyme testing, which involved screening with the Immunetics C6 Lyme ELISA kit (Oxford Immunotec), with equivocal and positive samples reflexed to the Viramed Biotech AG- Borrelia B31 IgM and IgG Virastripe Western blot assay (Trinity Biotech, Ireland). The samples were additionally tested using the BioPlex 2200 Lyme Total assay. When there was discrepancy between the BioPlex 2200 Lyme Total and the C6 ELISA, samples were subjected to Western blot if it had not been previously performed. After evaluation, the BioPlex 2200 Lyme Total assay was implemented as the routine screening test, in place of the C6 ELISA. Positivity rates for the screening assays and the corresponding Western blots were calculated for equal periods of time both pre and post BioPlex 2200 implementation.

Results: 78 samples were positive, and 325 samples were negative on both the Lyme Total and the C6 ELISA. Of the 78 positive samples, 62 we positive by either an IgM or an IgG Western blot. 46 samples screened positive using the C6 ELISA and negative on the Lyme Total; 5 of these samples were positive for IgM only via Western blot. 15 samples screened positive using the Lyme Total and negative using the C6 ELISA; 3 of these samples were positive for IgM only and 1 was positive for both IgM and IgG via Western blot. When the standard two-tiered algorithm was used to

interpret the results, the Lyme Total assay had a sensitivity of 92.96% and a specificity of 93.13%. The positive and negative predictive values of the test were 70.97% and 98.65%.

Conclusion: We found the BioPlex 2200 Lyme Total assay to have comparable sensitivity and better specificity than the C6 ELISA when Western blot was used to confirm positive results. Once implemented, the BioPlex 2200 Lyme Total assay helped to streamline the workflow in our laboratory. In the future we would like to examine the feasibility of using the multiplex BioPlex 2200 Lyme Total assay as a component of a modified two-tiered Lyme testing algorithm.

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Multiplex Immunoassay for Specific Human IgG Antibody Detection of Zika Virus Exposure and Differentiation from Other Flavivirus Infection

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Background: Zika virus (ZIKV) is a mosquito-borne flavivirus and caused the congenital Zika Syndrome. Nucleic acid amplification tests (NAATs) are the preferred method of diagnosis of confirming the infection in Zika virus disease. For non-pregnant persons with clinically compatible illness, ZIKV NAATs should be performed on serum collected ≤ 7 days after symptom onset. For symptomatic persons with possible exposure to dengue virus (DENV) and ZIKV, a positive NAAT result typically provides evidence of acute infection. Because the levels of virus in blood is transient and declines over time and possible inaccurate reporting of dates of illness onset, a negative NAAT result does not exclude ZIKV infection. Therefore, immunoassays play an important role in detecting the exposure to ZIKV in NAAT-negative serum specimens collected > 7 days after onset of symptoms. Five serologic assays are available for detection of anti-ZIKV IgM class antibodies with U.S. Food and Drug Administration emergency use authorization. In November 2019, CDC issued a testing guidance informing that in samples collected from asymptomatic and asymptomatic pregnant women Zika virus serologic testing is not recommended. This guidance was given because Zika IgM antibodies can persist for months to years following infection and ZIKV IgM antibodies might not indicate a recent infection. There is notable cross-reactivity between DENV IgM and ZIKV IgM antibodies in serologic tests. Antibodies generated by a recent DENV infection can cause the ZIKV IgM to be falsely positive. We developed a arbovirus multiplex detection of ZIKV specific human IgG and differentiate from many flavivirus infections. **Methods**: We developed a multiplex fluorescent microsphere array immunoassay for detection of ZIKV specific IgG and differentiating from other flavivirus infections. Our multiplex assay simultaneously measures IgG antibodies to ZIKV, four serotypes of DENV, West Nile virus, yellow fever virus, Japanese encephalitis virus, Saint Luis encephalitis virus, Usutu virus, Powassan virus and tick-borne encephalitis virus. This capture antigen indirect immunoassay can be carried out in two hours using microtiter plate format and is amenable to automation. This can also be configured to perform high sample throughput testing. We evaluated the multiplex assay performance using more than 200 well characterized blood samples. **Results:** Multiplex assay was able to clearly differentiate primary ZIKV exposure from secondary flavivirus infection. The assay was found out to be 100% specific with samples collected in a longitudinal set of 110 samples. We also developed a reflux testing assay format for the samples that were tested positive to many viral antigens and designated as secondary flavivirus exposure. Our reflux testing used a sample processing step prior to performing the multiplex assay, thus significantly increased the ZIKV IgG antibody detection specificity. **Conclusion:** We developed a multiplex ZIKV specific IgG antibody test that can clearly detect primary ZIKV and differentiates from other flavivirus exposure. We also developed a sample processing step that provides highly specific ZIKV IgG antibody detection in the blood sample. These tests have great potential ZIKV sero-surveillance and also for use for testing non-symptomatic and symptomatic pregnant women for ZIKV exposure.

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Evaluation of Investigational Modified Two-Tier Lyme Disease Testing Algorithms Against CDC-Recommended Algorithms in a Well-Characterized US Population

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Background: Lyme borreliosis is a tick-borne disease caused by the *Borrelia burgdorferi* sensu lato complex of bacteria. We evaluated the performance of investigational modified two-tier (MTT) Lyme testing alternatives to the standardized two-tier (STT) testing algorithm and the recently FDA-cleared MTTs. **Methods:** The MTT alternatives investigated consist of the FDA-cleared BioPlex 2200 Lyme Total test run as a 1st-Tier test followed by either the BioPlex 2200 Lyme IgM and BioPlex 2200 Lyme IgG tests (under development) run as the 2nd-Tier, or the bioMérieux VIDAS Lyme IgG II and Lyme IgM II tests run as the 2nd-Tier. The performance of these MTT combinations was evaluated using a 280-member panel of well-characterized Lyme positive and negative control samples from the CDC Lyme serum repository. **Results:** The MTT using the BioPlex2200-only assays improved overall 2-Tier sensitivity when compared to matched STT results (91.1% vs. 76.7%, respectively) and the three other MTT alternatives (84.4% - 87.8%). This higher sensitivity was achieved while maintaining a high level of 2-Tier specificity (98.9%). When compared to the newly cleared Zeus's MTTT-1 and MTTT-2 assay combinations, the investigational BioPlex 2200 only MTT displayed a higher overall 2-Tier sensitivity (91.1% vs. 84.4% and 87.8%, respectively) and specificity (98.9% vs. 96.8 and 98.4%, respectively), as well as a noticeably improved ability to detect early Lyme infections (86.7% vs. 76.7 and 81.7%, respectively; Stage I, EM positive). **Conclusion:** The investigational MTT using a combination of FDA cleared and under-development Bio-Rad Lyme assays was the most sensitive assay combination tested. Additionally, the BioPlex 2200 MTT was also the most specific MTT testing algorithm investigated.

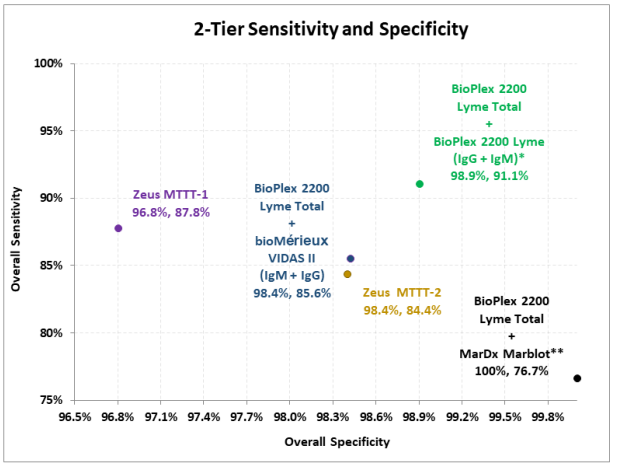

Figure 1. Overall 2-Tier Sensitivity and Specificity Comparisons of Lyme testing algorithms

*BioPlex 2200 Lyme IgG and BioPlex 2200 Lyme IgM assays are in development.
**MarDx Marblot test results were provided by the CDC; all other test results were tested at Bio-Rad Laboratories.

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Epidemiological Profile of Hepatitis B Virus Carriers in a Clinical Analysis Laboratory

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Background: Hepatitis B is a serious public health problem in Brazil and worldwide. The disease has a high potential for chronification and is one of the main causes of cirrhosis and hepatocellular carcinome, causing high costs to the health system. Although the prevalence of the disease has been decreasing since the introduction of the hepatitis B virus (HBV) vaccine in 1988, between 1999 and 2017, 218,257 cases were reported in Brazil. Approximately 10% of cases are concentrated in the northeast region. **Objective:** This study aimed to trace the epidemiological profile of HBV carriers from a clinical analysis laboratory. **Method:** A cross-sectional study was conducted to evaluate all patients with measurement of HBsAg performed in a clinical analysis laboratory, in northeast region of Brazil, that is part of a largest network of clinical analysis laboratories, between January to December 2018. Through the Business Intelligence tool, individuals with positive results for HBsAg serology from the database of the year 2018 were identified. Patients were categorized by gender, age group and origin. HBsAg measurement was performed by electrochemiluminescence on Cobas 6000 Roche platform. **Results:** Of the total of 152,144 patients who underwent blood collection at this laboratory in 2018, 16,123 (10.5%) underwent HBsAg serological testing. Of these, 63.77% are female. Eighty-two individuals (0.5%) had a positive result, 52,4% female. The age group from 30 to 39 years old was the most affected in the whole group, however, when separating the groups, the age group

most affected in males was 50 to 59 years old. Among the male who underwent the test, positive serology was found in 0.67% of the cases and in the female group, the positivity was 0.42%. Regarding the place of origin, most individuals (64.63%) came from the city where the laboratory was located, 35.36% came from nearby locations and 6.09% unknown origin. **Conclusion:** Knowing the silent evolution and the high impact on the health of HBV infection, early laboratory diagnosis is important for the identification of cases and to contribute to tracing the epidemiological profile of each location. This research may be useful to public health managers to support the development of prevention strategies, intensifying population campaigns in order to reduce the prevalence of hepatitis B infection.

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Emergency Validation of SARS-CoV-2 Virus Qualitative Detection by RT-qPCR in Nasopharyngeal Swabs Samples: Preliminary Results

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Background: An outbreak of respiratory illness caused by a novel coronavirus (SARS-CoV-2) first identified in Wuhan has spread internationally. A reliable laboratory diagnosis is mandatory. In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions. The aim of this study was to validate an automated RT-qPCR workflow for qualitative detection of SARS-CoV-2 virus in nasopharyngeal swabs samples. **Methods:** This validation enrolled 60 nasopharyngeal swabs samples collected from healthy volunteers on Cobas® PCR media (≤40% Guanidine hidrocloride) using polyester swabs. The PCR media was chosen to virtually inactivate the virus and preserve the sample RNA. WHO and CDC interim RT-qPCR protocols for SARS-CoV-2 detection were used. Each protocol described three primers/probes sets for SARS-CoV-2. WHO assays was abbreviated as N, E and RdRP and CDC were abbreviated as N1, N2 and N3. Each viral primer/probe set (FAM) were multiplexed with a human RPP30 gene primer/probe set (HEX) and with an artificial RNA sequence primer/probe set (CY5). All nasopharyngeal swabs samples were submitted to the six assays to check the generation of false positive results. A synthetic dsDNA molecule comprising all SARS-CoV-2 target sequences preceded by a T7 promoter were in vitro transcribed into its RNA form. This SARS-CoV-2 synthetic RNA was quantified and known copy number was spiked into negative swabs to construct positive samples. The RT-qPCR workflow was executed on a Flow Flex Solution (Roche) using manufactor recommended reagents. The artificial RNA sequence was added into samples during the nucleic acids extraction. Approved test were apply to a 1:10 (from $1.48x10^8$ to $1.35x10^2$ copies/reaction) and 1:2 (from $2.65x10^4$ to $2.02x10^{-1}$ copies/reaction) serial dilutions of the synthetic RNA in order to calculate the assay linearity and the limit of detection (LOD) by linear regression and probit regression analysis, respectively. The assays trueness was evaluated by the agreement between expected and observed results for all samples with RNA load > 100 copies/reaction. Negative samples were included in each experiment. **Results:** Consistent false positive results in healthy volunteers were observed for N, N2 and N3 primer/probe sets and they were eliminated from the validation. N1, E and RdRP assays showed linear response from $1.48x10^8$ to $1.35x10^2$ copies/PCR: R^2 of 0.999, 0.995 and 0.991 and amplification efficiency of 93.4%, 86.32%, and 116.5%, respectively. LOD for N1, E and RdRP assays was 22.0 (95%CI 15.7-42.7), 241.0 (95%CI 158.0-571.4) and 303.8 (95%CI 228.9-544.0) copies/PCR, respectively. The comparison between obtained and expected results in samples with >100 copies/reaction revealed total agreement of 100% (95%CI 97.9-100%), 98.3% (95%CI 95.1-99.4%) and 97.1 (95%CI 93.5-98.8%). Negative samples always returned negative results in N1, E and RdRP assays. **Conclusion:** RdRP, E, and N1 assay are suitable for SARS-CoV-2 detection in nasopharyngeal swabs. Among them, the N1 was the most sensitive resulting in higher trueness and should be used in daily routine. E assay showed the second best performance and can be used as confirmatory testing. The main limitation of this validation is that the real virus could not be tested yet.

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A TRAIL/IP-10/CRP Host-Protein Assay for Distinguishing between Bacterial and Viral Infection has Potential to Reduce Antibiotic Overuse in Adults with Lower Respiratory Tract Infection (LRTI): Preliminary Results from the OBSERVER Clinical Study

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Background: Lower respiratory tract infection (LRTI) is a clinical challenge due to the difficulty in pathogen sampling and the often-indistinguishable symptoms of bacterial and viral etiologies. This diagnostic uncertainty leads to antibiotic misuse, potentially exposing the patient to unnecessary side effects and contributing to antimicrobial resistance. Previous studies showed that a host-protein assay comprising TNF-related apoptosis induced ligand (TRAIL), interferon gamma induced protein-10 (IP-10) and C-reactive protein (CRP) accurately differentiates between bacterial and viral infections. Here we examine the potential of the assay to reduce antibiotic misuse in adult patients with suspected LRTI.

Methods: Adults aged > 18 years with suspected LRTI were recruited in a prospective study at three emergency departments in Israel (OBSERVER; H2020 grant #684589; NCT03011515). Infection etiology was adjudicated by majority decision of three experts based on clinical, laboratory, radiological and follow-up data. The host-protein assay (ImmunoXpert™, MeMed) gives three possible outcomes: viral, bacterial or equivocal; equivocal is a valid result that does not provide clear-cut etiology determination.

Results: 414 adults with suspected LRTI were included in this preliminary analysis (mean age 54.6) Clinical syndromes included: 29% pneumonia, 27% upper respiratory tract infection, 17% acute bronchitis, 6% asthma or chronic obstructive pulmonary disease exacerbation and 13% unspecified LRTI or viral infection. 190 were assigned viral etiology and of these, 104 were given antibiotics, representing an overuse rate of 55%. Adoption of the host-protein assay would lead potentially to only 30 out of the 190 viral patients being given unwarranted antibiotics (Table); a greater than 3-fold reduction in antibiotic overuse (p-value < 0.0001).

Conclusions: This study indicates that timely host-protein assay results may aid in determining infection etiology and reduce unwarranted antibiotics among adults with suspected LRTI. Use of this new assay could help improve adherence to antibiotic prescription guidelines.

Host-protein assay result	Antibiotic decision observed in study		Number of adults given antibiotics if host-protein assay adopted
	Abx-	Abx+	
Bacterial	4 (4.7%)	8 (7.7%)	12
Equivocal	13 (15.1%)	18 (17.3%)	18*
Viral	69 (80.2%)	78 (75.0%)	0

*Physician decision about antibiotics would be unchanged when result is equivocal

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Point of Care Testing

A-291

Time-of Day and Day-of Week Patterns in Use of Epoc Point-of-Care Blood Gas Measurements within Intensive Care Units at a University Hospital

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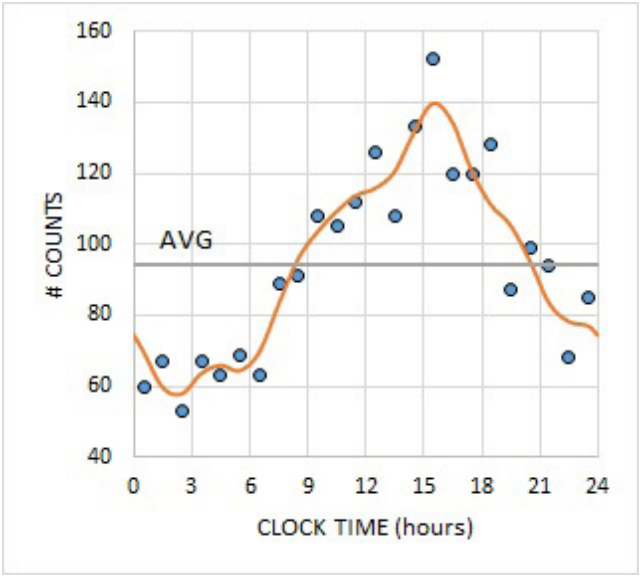
Background: Central hospital laboratories usually exhibit fixed patterns of variation in time-of-day (TOD) and day-of-week (DOW) rates of inpatient sample submissions. Patterns for inpatient point-of-care testing (POCT) have never been examined at our institution, however. POCT using Epoc analyzers (blood gas, electrolytes, metabolites, or BGEM) was introduced into intensive care units (ICUs) at our hospital primarily for emergency use. As such, it was anticipated that both TOD and DOW patterns of rates of use in ICUs should have little variation from a running average. We examined this premise by review of BGEM data recorded over a 1-year interval. **Methods**: Data were retrieved from the POCT information system (RALS) for patient sample BGEM measurements from among all five full-time ICUs in our hospital over the interval July 2018-June 2019. Analyses of TOD and DOW data were performed using Python and Excel programming. **Results**: Data were from 2267 BGEM measurements. Utilization was very conservative, with daily rates of use among individual ICUs ranging from 0.41 to 2.67 measurements/day. There was a distinct TOD pattern in the combined measurements (see Figure). Among one-hour segments, the high to low ratio of rates was 2.87. A contiguous 10-hour time segment (0900-1900) exhibited continuous above-average rates, comprising 53% of results within 42% of a 24-hour cycle. Combining TOD and DOW analysis, TOD variation was almost exclusively due to variation during weekdays (Mon-Fri) rather than weekends (Sat-Sun). Moreover, per-day utilization was much greater on weekdays than on weekends, with ratio = 1.82. **Conclusions**: Counter to expectation, patterns of Epoc/BGEM utilization within ICUs showed great variation both in TOD and DOW. Potential causes of variation may include TOD- and DOW-dependence of transfers, census, certain procedures, and testing preference among staff. These data add incrementally to general literature regarding TOD and DOW variation in numerous aspects of hospital practice.



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Performances of Processed Blood Materials from *In Vitro* Glycation for Hemoglobin A_{1c} Testing

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Background: Hemoglobin A_{1c} (HbA_{1c}) is a glycated hemoglobin in which glucose is bounds specifically to the N-terminal valine of the hemoglobin β chain. Quality control materials for HbA_{1c} at a high level are difficult to prepare from patient blood samples due to limitations in mass production as well as the instability of glycated hemoglobin. This study evaluated performance of processed blood materials from *in vitro* glycation in HbA_{1c} testing. **Methods:** A proper blood sample from a blood bank which A_2A on Hemoglobin typing and $HbA_{1c} \leq 5.0\%$ was used for glycation. *In vitro* glycations for glycated hemoglobin was performed by suspending blood samples into 0.85% normal saline at pH 7.4. The sedimentation of RBC was separated and incubated with 150 mM of D-glucose in pH 7.4 phosphate buffer at 37 ^{o}C for 6 hours to produce HbA_{1c} approximately 7.0% to 8.0% and for 9 hours to produce HbA_{1c} more than 8.0%. After incubation, processed blood materials at particular concentrations of HbA_{1c} were aliquoted and kept in citrate-phosphate-dextrose-adenine-1(CPDA-1) solution with 7.2 mmol/L of glucose at 2-8 ^{o}C. HbA_{1c} in processed blood materials from *in vitro* glycation was measured by the secondary reference analyzer based on an immunoassay. The homogeneity and stability of HbA_{1c} were investigated by following ISO 17034 and ISO Guide 35 guidelines. **Results:** Blood samples with $HbA_{1c} \leq 5.0\%$ could be used for *in vitro* glycations to produce HbA_{1c} for 8.8±0.12 % at 6 hours of incubation and for HbA_{1c} 9.2±0.20 % at 9 hours of incubation. The homogeneity of HbA_{1c} in processed blood materials was accepted with $F < F$ critical by using ANOVA single factor analysis. HbA_{1c} in process blood materials was stable for at least 109 days with $t < t$ critical by using statistics following ISO Guide 35 guidelines. HbA_{1c} was significantly increased ($p < 0.05$) by 8.60% at 6 hours of incubation and by 6.43% at 9 hours of incubation when compared to the amount at baseline. **Conclusion:** Processed blood materials for HbA_{1c} testing with an immunoassay could be prepared from *in vitro* glycation by incubation with D-glucose solution at pH 7.4 for 6 to 9 hours. Processed blood materials represented good performance with sufficient homogeneity and HbA_{1c} was stable for at least three months. Thus, these processed materials from *in vitro* glycation may be useful as QC material for HbA_{1c} testing.

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Point-of-Care Lactate Testing: Achieving Target Turnaround Times with Acceptable Performance Specifications

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Background: Rapid measurement of blood lactate is important for evaluating acid-base status and detecting tissue hypo-perfusion in critically ill and septic patients. International consensus-based guidelines recommend that lactate measurement results be available with a turnaround time (TAT) of 30 min from specimen collection to result. Our hospital's sepsis protocol requires a TAT of 60 min from order to result, and our hospital's acute care laboratory experienced difficulty in meeting the TAT goal for lactate orders in 2019. Only 76% of lactate orders met the TAT goal from May-Oct. 2019 (n=385 orders). Therefore, we evaluated lactate point-of-care (POC) testing using the Nova Biomedical® StatStrip Lactate Meter as a potential solution to improve TATs. A method validation study was conducted to benchmark the performance of the Nova Lactate Meter vs. the Radiometer® ABL90 blood gas analyzer. A key metric for comparison was whether clinical decisions would change based on the consistency of results ≥2.1 mmol/L (critical action value for sepsis protocol initiation) between the two instruments.

Methods: The Radiometer ABL90 blood gas analyzer was used as the reference instrument and two Nova lactate meters were validated concurrently. Precision was determined over 20 days with four measurements per day using two levels of commercially available control material (0.7 and 5.9 mmol/L). The method comparison study was conducted using 40 remnant patient anticoagulated whole blood specimens. The

timing between the analysis on each instrument was 15-20 seconds with the samples run first on the ABL90 and then the Lactate Meter. Statistical analysis was conducted using EP Evaluator 11.1.0.26 (Data Innovations).

Results: The Lactate Meter CVs in the precision study using level 1 and 2 of the control material were 13.8% and 9.5%, respectively. The assay was linear over a range of 0.3-20.0 mmol/L. These results were within acceptable performance limits. However, significant performance deviations were observed in the method comparison study. The Lactate Meter results had an average bias of -17.6%. Nova offers the implementation of a software-based offset value to correct biased results. Based on the linear regression analysis of the method comparison data, an optimal offset of y-intercept = 0.3 was applied. The precision, linearity and method comparison studies were repeated after applying the offset. Without implementing the offset, 8% of the samples in the method comparison study had lactate levels <2.1 mmol/L when analyzed using the Lactate Meter, but ≥2.1 mmol/L when analyzed using the ABL90. Implementing the offset improved the percentage of discrepant results to only 7%. These discrepant results would have altered clinical management decisions.

Conclusion: Although implementing a software-based offset value on the Lactate Meter marginally improved the bias, the results from the method comparison study rendered the device unacceptable for use as a POC solution to improve the TAT for lactate results at our hospital. As an alternative POC solution for lactate testing, to improve TATs, we initiated training of OR staff on ABL90 use and we improved laboratory workflows to decrease the time between specimen collection and analysis.

A-295

Rapid and Accurate Point-of-Care Detection of Lethal Amatoxins from Mushrooms and Urine

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Background: With the continued emergence and spread of amatoxin-containing mushrooms globally, there is need for a rapid, accurate, and easy-to-use technology to both identify deadly poisonous mushrooms and to improve clinical diagnosis of mushroom poisonings in humans as well as in animals. Globally, mushroom poisonings cause about 100 human deaths each year, with thousands of people requiring medical assistance. The mushroom poison that causes the most deaths is amanitin (or amatoxins α-, β-, and γ-amanitin, AMA). Current methods to sensitively and selectively detect these toxins are limited by the need for expensive equipment, or they lack accuracy due to cross-reactivity with other chemicals found in mushrooms. **Objective:** To validate a competitive-based lateral flow immunoassay (LFIA) for the rapid, simple, portable, selective, and sensitive detection of amatoxins. **Methods:** This study was performed by testing wild mushrooms and urine samples for the presence or absence of amatoxins. This test does not cross-react with toxins from other known poisonous mushrooms or typical components in urine. Mushroom samples consisted of both dried and fresh specimens, and all were identified to species by expert mycologists. Mushrooms were extracted in a saline buffer, inverted for <1 min, and a small aliquot of the sample was applied to the LFIA test strip. Human urine samples were fortified to recapitulate previously identified amatoxin concentrations from an exposure study. Dog urine samples were obtained from suspected mushroom intoxications as well as control samples from healthy and sick animals. Urine samples were directly applied to the LFIA test strip without sample preparation. **Results:** Our LFIA test strip detects as little as 10 ng/mL of α-AMA or γ-AMA in mushroom extracts or in urine samples. For mushroom sampling, the entire method including extraction and detection can be completed in approximately 10 minutes. From testing 110 wild mushrooms, the LFIA accurately identified 6 out of 6 (100%) species that were known to contain amatoxins, while other poisonous mushrooms known not to contain amatoxins tested true negative for amatoxins. For urine sampling, results were revealed in approximately 10 minutes. Using the LFIA test, analysis of both fortified human urine samples and urine samples from intoxicated dogs, correlated well with liquid-chromatography mass spectrometry (LC-MS) methods. The accuracy of the LFIA in identifying amatoxin positive urine samples ranged from 78.9-94.6% with limited sample numbers (N=38 and N=96, respectively). **Conclusions:** The data illustrates excellent analytical performance of the LFIA for the rapid, sensitive, selective, and accurate detection of amatoxins. This assay can be used to address the shortcomings of current clinical diagnostics (speed, portability, cost), holding high promise to identify fatal mushroom poisonings sooner. In addition, this LFIA can be used to aid mycologists and clinicians to quickly identify amatoxin-containing mushrooms.

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A-296

A Significant Drug Interference Observed on the i-STAT Point-of-Care Creatinine Method Due to Hydroxyurea

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Background: Point-of-care (POC) creatinine has gained use for the evaluation of renal function immediately before administration of contrast for imaging procedures. The rapid turn-around time and good correlation with reference methods make POC creatinine measurement an attractive option in this clinical setting.**Introduction:** We present the case of a 61-year-old man with history of metastatic prostate cancer with no history of kidney dysfunction whose pre-imaging measured creatinine on the i-Stat analyzer was 4.4 g/dL. Confirmatory testing in our core laboratory (Siemens Advia 1800) gave a creatinine of 0.92 g/dL. Repeat testing several weeks later showed similar results (POC: 4.4 and core laboratory 0.95). We suspected an interference, possibly a drug effect. **Methods:** In the i-STAT POC method, creatinine is hydrolyzed to creatine, which is then hydrolized to sarcosine. Sarcosine is subsequently oxidized by the enzyme sarcosine oxidase to produce hydrogen peroxide. Hydrogen peroxide then reacts at a platinum electrode to produce an electric current proportional to the creatinine level in the blood sample. Our core laboratory Advia method is based on the kinetic Jaffe alkaline picrate reaction. R**esults/Discussion:** While our POC i-STAT creatinine testing has been shown to be comparable to reference methods in terms of accuracy, the i-STAT method employed in this patient's case is subject to significant interference by several commonly used drugs including acetaminophen, ascorbate, hydroxyurea, and creatine. On review, our patient was prescribed hydroxyurea as part of a clinical trial. We tested pooled plasma specimens containing hydroxyurea and noted an increase in i-STAT creatinine results. The manufacturer recommends use of a suitable alternative method when drug interference is suspected. **Conclusion:** Some commonly used medications can interfere with the measurement of creatinine on the i-STAT method. Therefore, it is important that the clinician and laboratory director are aware of these interferences and that an alternate reference method is used to confirm clinically unexpected results.

A-299

Pilot Interference Evaluation of the Lab 001™

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Background: Important diagnostic and therapeutic decisions are routinely made based on glycated hemoglobin (HbA1c) measurement, which is an important indicator of glycemic control in diabetics. The Lab 001, a novel A1c measurement device from ARKRAY, is not for sale in the US. The device measures HbA1c (NGSP %) in human whole blood using modified capillary electrophoresis technology. The device features self-contained reagent cartridges that eliminate the need for device maintenance. The purpose of this study was to evaluate the interference of select drugs on the accuracy of A1c measurements by The Lab 001.

Methods: An interference study was performed per CLSI EP07-A2 *Interference Testing in Clinical Chemistry*. Fifteen (15) drugs with the potential to interfere with HbA1c test results were analyzed by spiking the interferent into two whole blood samples with HbA1c values of ~6.5% and ~8.0%. Three (3) replicates of each interferent test sample and solvent-only control sample were analyzed using The Lab 001 system. Trimethoprim and sulfamethoxazole were tested in combination as literature suggests a potential to interfere with HbA1c measurements exists when administered together.[1]

Results: No significant interference was observed at therapeutic levels up to the highest concentration of the fifteen (15) drugs / drug combinations tested: acetaminophen (240 mg/dL), acetylsalicylic acid (360 mg/dL), caffeine (48 mg/dL), dapsone (18 mg/dL), dopamine (72 mg/dL), glyburide (1.2 mg/dL), hydroxyurea (462 mg/dL), hydroxyzine dihydrochloride (36 mg/dL), ibuprofen (192 mg/dL), ribavirin (84 mg/dL), theophylline (36 mg/dL), trimethoprim & sulfamethoxazole combination (19.2 & 96 mg/dL), urea (5400.2 mg/dL) and zidovudine (36 mg/dL).

Conclusion: Pilot studies showed no significant interference from fifteen (15) drugs with the potential to interfere with HbA1c measurements at therapeutic levels. The Lab 001 system is a robust, safe, and accurate method for routine HbA1c measurement in laboratories. Further studies evaluating its performance is required.

[1] https://www.ncbi.nlm.nih.gov/pubmed/12905153/

Drugs Tested	
Interferent	**Highest concentration without Interference (mg/dL)**
Acetaminophen	240.0
Acetylsalicylic acid	360.0
Caffeine	48.0
Dapsone	18.0
Dopamine	72.0
Glyburide	1.2
Hydroxyurea	462.0
Hydroxyzine Dihydrochloride	36.0
Ibuprofen	192.0
Ribavirin	84.0
Theophylline	36.0
Trimethoprim & Sulfamethoxazole	19.2 96.0
Urea	5400.2
Zidovudine	36.0

A-300

Performance Evaluation of Creatinine, Blood Urea Nitrogen and tCO_2 using Whole Blood Point-of-Care Analyzers

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Background: GEM Premier ChemSTAT (Instrumentation Laboratory/IL, Bedford MA) is a new point-of-care (POC) system that provides rapid results for Na^+, K^+, Ca^{++}, Cl^-, glucose, creatinine (Crea), blood urea nitrogen (BUN), tCO_2, hematocrit, lactate, pH and pCO_2 from a single whole blood (WB) sample. i-STAT and Piccolo Express® (Abbott Laboratories, Chicago IL) are WB analysers intended for use in a clinical laboratory or POC location. The goal of this evaluation was to compare Crea, BUN and tCO_2 performance across these POC systems compared to the laboratory cobas (Roche Diagnostics, Risch-Rotkreuz, Switzerland) chemistry analyser.

Methods: Remnant WB samples from a local hospital were evaluated on GEM ChemSTAT, i-STAT and Piccolo Express. Plasma aliquots were tested with the cobas c311 analyzer for Crea, BUN and tCO_2. All samples ≤2 mg/dL Crea covering relevant Medical Decision Levels were used for analysis of Crea.

Results: WB results from GEM ChemSTAT demonstrate excellent agreement with the cobas enzymatic assays. Both i-STAT and Piccolo Express show acceptable performance for Crea, low mean bias for BUN and noticeable high mean bias for tCO_2 compared to laboratory assays from cobas. The margin of error calculated as 95% confidence interval (CI) of mean bias is smallest for GEM ChemSTAT for all analytes, demonstrating higher precision of the three POC systems evaluated. The results from Bland-Altman analysis and the mean bias along with 95% CI estimated per analyte are summarized in Table 1.

Conclusion: GEM Premier ChemSTAT shows strong correlations with the laboratory comparative method for all the analytes. Both i-STAT and Piccolo Express' analytical performance shows significant bias for BUN and tCO2 against cobas c311 assays. With its operational simplicity, reliability and quicker turnaround time, the ChemSTAT can provide lab-quality WB chemistry panel results at the POC to expedite treatment and improve patient management.

Table 1. Bland-Altman Analysis Results for the WB POC Systems vs. Laboratory Results

Analyte	Analyzer	N	Mean Bias	95% CI	Sample Range
Crea (mg/dL)	GEM ChemSTAT	1028	-0.039	-0.044, -0.034	0.41 - 1.99
	i-STAT	302	-0.052	-0.060, -0.043	0.41 - 1.99
	Piccolo Express	80	-0.017	-0.050, 0.016	0.38 - 1.85
BUN (mg/dL)	GEM ChemSTAT	1168	0.55	0.47, 0.62	4.7 - 99.2
	i-STAT	337	-2.04	-2.43, -1.64	5.7 - 106.6
	Piccolo Express	103	-2.39	-2.73, -2.05	5.4 - 85.3
tCO_2 (mmol/L)	GEM ChemSTAT	357	-0.48	-0.57, -0.39	15.7 - 28.6
	i-STAT	37	3.15	2.62, 3.68	19.1 - 42.3
	Piccolo Express	104	3.81	3.40, 4.03	5.4 - 28.6

A-301

An Impedance Platelet Aggregometer for Point-of-Care Testing of Platelet Hemostatic Function

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Background: Platelets are vital contributors to hemostasis, providing protection from excessive bleeding in such as may be seen from trauma or following surgical or interventional procedures. While platelet function testing traditionally requires laboratory processing of blood to yield platelet-rich plasma (PRP), whole blood (WB) systems have been advocated for point-of-care (POC) application. Current WB systems have limitations as to size, protocol complexity, operator requirements or readout diagnostic interpretation. We developed a versatile, easy-to-use POC diagnostic device for rapid platelet function assessment. The MICELI (MICrofluidic, ELectrical, Impedance) aggregometer is a first-generation device for assessment of platelet aggregation in WB or PRP using 250 µL of the sample with results available within 10 min. Here, we validate the precision and accuracy of the MICELI platform, as a precursor to the development of a second-generation device, by measuring aggregation response to traditional platelet agonists, assessing potential interferences, e.g. platelet count (PC) and hematocrit (HCT), and comparing results to a commercial impedance aggregometer. **Methods:** Whole blood from 30 healthy volunteers was collected via venipuncture and anticoagulated with hirudin. Platelet aggregation in WB and PRP was induced by ADP, collagen, TRAP-6 or epinephrine; the aggregation parameters, e.g. maximal amplitude (Amax) and area under the curve (AUC) were measured. MICELI precision was evaluated by redundant testing of single donor WB and PRP samples. Accuracy was assessed as compared to the Multiplate® Analyzer (Roche, Switzerland), using ADP. HCT was quantified using the microhematocrit method; PC was measured by the Z1-Coulter particle counter (Beckman, USA). **Results:** Characteristic impedance curves that graphically trace platelet aggregation on the MICELI electrodes were observed in WB and PRP following stimulation by the agonists. Repeatability (CV%) of the aggregation parameters ranged from 21% to 24% (intra-donor) and from 6% to 22% (inter-donor) demonstrating the highly consistent performance of the MICELI platform with different agonists. The MICELI showed less sensitivity to PC in WB compared to PRP (R^2 = 0.7661 vs. 0.9862, respectively) across the physiological PC range of 150-400,000 platelets/µL. HCT did not significantly affect aggregation values within the range of 0.35-0.45 (R^2 = 0.463). The comparison of the MICELI and the Multiplate® showed a good correlation of aggregation parameters, with R^2 = 0.920 and 0.980 for AUC and Amax, respectively. **Conclusions:** Using impedance measurement of whole blood, the MICELI aggregometry platform demonstrated repeatable aggregation response to conventional agonists with comparable results to a commercial aggregometer. A second-generation device that includes a self-filling cartridge with strict volume control, the reagent agonist in biostable form preloaded in the cartridge and a multi-well cartridge to accommodate the POC test setting is currently under development. This novel device offers potential for POC assessment of platelet function in a number of critical care settings and thus delivery of personalized hemostatic interventions.

A-302

Impact of a Local Hospital Closure to Increase Emergency Department (ED) Encounters: Evaluation of Rates of Measurements and of Results Distributions for ED Point-of-Care Glucose Testing

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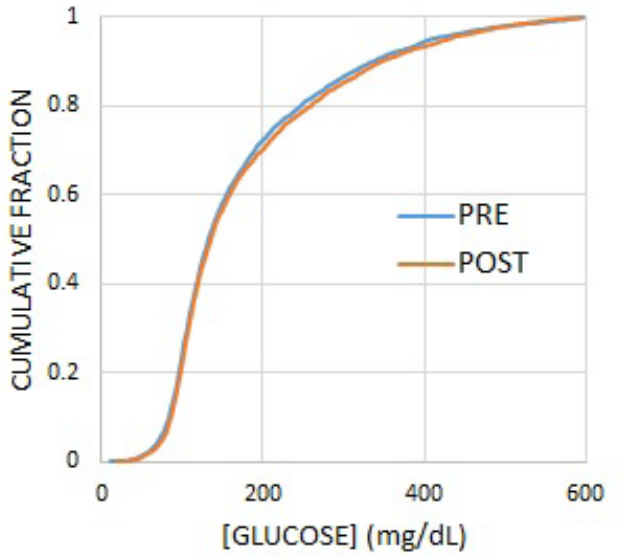
Background: A major event in Philadelphia in 2019 was the closure of Hahnemann Hospital, a primary university-affiliated hospital located in Center City. One anticipated effect of this closure was increased demand for emergency department (ED) services in surrounding hospitals. We investigated the effect from the perspective of point-of-care testing (POCT) for glucose in our ED, by retrospective analysis and comparison of pre- and post-closure rates of testing and characteristics of the overall patient population distribution of results. **Methods:** The closure of Hahnemann's ED occurred on 8/16/2019. POCT glucose measurements were extracted from the RALS laboratory middleware for two 91-day intervals pre-closure (2/15/19-5/16/19) and post-closure (8/16/19-11/14/19), each inclusive of the same numbers of each day-of-week. Data were analyzed using Excel and Origin software. **Results:**

ED POCT glucose measurements in the post-closure interval (n=4597) were increased by 22.6% compared to the pre-closure interval (n=3750). As a proxy for ED encounters, numbers of POCT glucoses indicated that the closure significantly impacted our ED. Pre- and post-closure results distributions were essentially indistinguishable (p>0.9, r2>0.99) (see *Figure*), including identical rates of critical values ([glucose]>500 mg/dL: 2.00% (pre-closure) vs. 2.00% (post-closure)). The general stability of analyte cumulative distributions across time is remarkable. Such data are likely to change only due either to a change in characteristics of the patient population or a systematic error in measurement. Documentation of such distributions is therefore useful for quality assurance purposes. It was thus worthwhile to document the current distribution after such a significant change in patient numbers. **Conclusion:** A local hospital closure was followed by a significant increase (+23%) in rate of POCT glucose measurements in our ED. Such an increase will affect the budget required for POCT supplies. As a matter of quality assurance, ED POCT glucose results distributions were found to be unchanged between pre- and post-closure intervals.



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GEM Premier ChemSTAT Evaluation at Wythenshawe Hospital

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Background: As a new point-of-care (POC) system, GEM Premier ChemSTAT (Instrumentation Laboratory/IL, Bedford, MA) provides rapid results for Na^+, K^+, Ca^{++}, Cl^-, glucose, creatinine (Crea), blood urea nitrogen (BUN), tCO_2, hematocrit, lactate, pH and pCO_2 from a single whole blood (WB) sample. The goal of this evaluation was to compare WB analytical performance and usability of the ChemSTAT analyser in a POC setting, Accident & Emergencies Department (A&E), to the current laboratory Crea and BUN assays, and other assays deployed at the POC at Wythenshawe Hospital.

Methods: Remnant WB samples from the A&E Rapid Assessment Area sent for testing on the GEM Premier 5000 (IL) were immediately evaluated on the GEM Premier ChemSTAT analyser (IL). As a comparative method, plasma aliquots from the same samples were assayed on a laboratory cobas 8000 (Roche Diagnostics, Risch-Rotkreuz, Switzerland) analyser for Crea (enzymatic assay), BUN, Na^+, K^+, and Cl^-. WB sample results from GEM Premier 5000 (IL) were also used for correlation.

Results: WB Crea, BUN and all other analyte results from the GEM Premier ChemSTAT correlated well with those from plasma tested on the cobas or WB samples on GEM 5000 across the tested sample ranges. Table 1 summarizes the regression results. Analyte biases (at 95% CI) were estimated at each medical decision level (MDL) per analyte and were all within manufacture's claims. Due to the pre-analytical error in transferring plasma samples, tCO_2 was not included in this study.

Conclusion: Strong correlations were observed between the GEM Premier ChemSTAT assays compared to the laboratory and current POC assays for all the analytes. With its operational simplicity, reliability and fast turnaround time, the ChemSTAT can provide lab quality WB chemistry panel results at the POC to expedite time to treatment, improving patient management and quality of care.

Table 1. Method Correlation Results for the GEM Premier ChemSTAT WB Assays *vs.* Comparative Methods

ChemSTAT Analyte	Roche cobas 8000 (N=96)				GEM Primer 5000 (N=102)			
	Slope	Intercept	R	Sample Range	Slope	Intercept	R	Sample Range
BUN	1.028	0.3674	0.996	1.9 - 42.08 mmol/L	n/a			
Crea	1.026	-3.531	0.996	40 - 868 umol/L	n/a			
tCO_2	Not included in correlation study				n/a			
Na^+	1.051	-9.627	0.956	125 - 149 mmol/L	1.012	-1.44	0.913	122 - 147 mol/L
K^+	1.000	0.000	0.991	3.1 - 5.6 mmol/L	1.000	0.000	0.983	3.0 - 8.3 mol/L
Cl^-	1.000	2.850	0.964	84.1 - 111 mmol/L	1.000	-1.00	0.967	88 - 113 mmol/L
iCa^{++}					0.900	0.101	0.875	0.87 - 1.49 mmol/L
Hct					0.946	2.99	0.974	23 - 59 %
Glu					0.955	-0.049	0.995	4.0 - 31.0 mmol/L
Lac					0.944	-0.1	0.991	0.6 - 5.5 mmol/L
pH					1.050	-0.36	0.970	7.21 - 7.57
pCO_2					1.043	-0.217	0.976	3.2 - 8.5 kPa

A-304

Development and Preliminary Validation of a Fluorescence-Based Cardiac Troponin I Immunochromatographic Assay Correlated Well with bioMerieux VIDAS Troponin I Ultra

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Backgroud: The bioMerieux VIDAS Troponin I Ultra is an enzyme-linked immunofluorescence assay. The VIDAS Troponin I Ultra is a sensitive diagnostic method for the early detection of MI and predicts increased risk for adverse events in patients with symptoms suggestive of acute myocardial infarction (AMI). The obvious advantage of point-of care tests (POCT) is its quick turn-around time (TAT). However, the assay performance of POCT seems not satisfied with clinical laboratories, such as its poor sensitivity and precision. We have been extremely focused to develop a rapid and

more accurate cardiac troponin I (cTnI) POCT assay for use in the emergence rooms and family doctors' offices. **Methods:** The format design of our assay was chosen as a sandwich immunoassay. The cTnI protein released into patients' blood streams is combined with a cTnI specific monoclonal antibody (clone: 19C7, HyTest, Finland) labeled with the fluorescent latex beads (FCEU002, Bangs Laboratories, USA), forming an antigen-antibody complex. This complex is then migrated to the test zone and captured with a cTnI monoclonal antibody (clone: 16A11, HyTest, Finland) which is pre-coated on the nitrocellulose membrane (1UN14ER100025NT, Stedim, Germany), yielding a fluorescent signal. The immunofluorescence analyzer is in-house manufactured (Hunan Yonghe-Sun Biotech, China). The in-house QC material (named as "ILCH") used in this study was previously reported (A-009, p.S3, 71th AACC Annual Scientific Meeting Abstracts, 2019). The correlation between our assay and VIDAS Troponin I Ultra was performed in which 99 clinical serum samples valued with VIDAS Troponin I Ultra (provided by The First Hospital of Changsha, Hunan, China) were measured by using our assay. **Results:** Our preliminary results of validation were shown that the limit of detection (LoD) is 0.1 ng/ml with a detection range up to 50 ng/ml. The within-run precision, in terms of inter-assay coefficient variations (%CV), was lower than 15%. Furthermore, the comparative study of our assay with VIDAS Troponin I Ultra was performed. We found that overall correlation between our assay and VIDAS Troponin I Ultra was pretty well (R^2=0.9858). **Conclusion:** In this study, we described a fluorescence-based immunochromatographic cTnI assay which is found well-correlated with the VIDAS Troponin I Ultra. The preliminary validation results imply that the antibodies used in both our assay and VIDAS Troponin I Ultra may recognize the same or similar epitopes on the cTnI antigen. Notably, our assay is a POCT platform which TAT is 15 minutes. Therefore, as the frontline screening test for AMI, our rapid and more accurate cTnI test kits would be utilized on ambulance and in emergency rooms. The further validation studies and assay optimizations are being conducted.

A-305

Development and Preliminary Validation of a Fluorescence-Based NT-proBNP Immunochromatographic Assay Correlated Well with Roche Elecsys proBNP II

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Background: Heart failure (HF) affects 1-3% of the general population and approximately 10% of the elderly. Healthcare spending for chronic HF accounts for 1-2% of total healthcare expenditure in developed countries. N-terminal pro-brain natriuretic peptide (NT-proBNP) has a longer half-life and better stability than brain natriuretic peptide (BNP), so NT-proBNP is more sensitive to early or mild HF. The POCT test significantly shortens the turn-around time (TAT), and wins treatment decision for critical emergency patients, such as HF. Treatment before admission of emergency patients is related to patients' rescue and recovery. In emergency room, doctor needs to perform emergency assessment and re-evaluation of patients to determine appropriate treatments. We have been extremely focused to improve and optimize our existing POCT platform, and develop a more accurate POCT-based NT-proBNP assay for use in the emergence rooms and family doctors' offices.

Methods: This assay is a sandwich immunoassay format, using a monoclonal capture antibody and a fluorescently-labeled monoclonal detecting antibody. First, the NT-proBNP protein in patients' blood samples is combined with the detection monoclonal antibody (clone: 4NT-15F11, HyTest, Finland) labeled with the fluorescent latex beads (FCEU002, Bangs Laboratories, US), forming an antigen-antibody complex. This complex is then migrated to the test zone by capillary force and captured with a NT-proBNP monoclonal antibody (clone: 4NT-16E6, HyTest, Finland), which is pre-coated on the nitrocellulose membrane (1UN14ER100025NT, Stedim, Germany) yielding a fluorescent signal. The immunofluorescence analyzer is in-house manufactured (Hunan Yonghe-Sun Biotech, China). The clinical serum samples measured with VIDAS Troponin I Ultra were provided by The First Hospital of Changsha, Hunan, China.

Results: The analytical sensitivity of this assay was 100 pg/mL with a detection range up to 25000 pg/mL. The within-run precision, in terms of inter-assay coefficient variations (%CV), was 12% at 298 pg/mL and 7% at 4500 pg/mL. Furthermore, the comparative study of our assay with Roche Elecsys proBNP II was performed, in which 111 clinical serum samples measured with Elecscys proBNP II were tested by using our test kits. The linear regression between our assay and Elecsys proBNP II was found quite well (r=0.971).

Conclusion: We described in this study an improved POCT-based diagnostic assay for NT-proBNP, which is found quite well-correlated with the Elecsys proBNP II. The results of our preliminary validation imply that the antibodies used in both our assay and Elecsys proBNP II may recognize the same or similar epitopes on the proBNP antigen. Thus, our POCT-based rapid NT-proBNP assay could be an ideal diagnostic test for HF for earlier use in the emergency rooms and family doctors' offices. Although our results seem to be encouraging, there are more assay optimizations required. Further validation studies are being continued and performed.

A-306

Pediatric Reference Intervals for Critical Point of Care Assays in the CALIPER Cohort of Healthy Children and Adolescents

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Background: Point-of-care testing (POCT) is being increasingly adopted to support clinical institutions globally, facilitating patient-centered care as well as rapid clinical decision-making in emergent situations. Despite its evolving role in laboratory medicine, normative pediatric data for critical care parameters (e.g. blood gas, electrolytes, metabolites) on POCT instruments is seriously lacking. Here, we aimed to address this evidence gap by establishing comprehensive reference standards for several critical care parameters on the Radiometer ABL90 FLEX Plus platform in the CALIPER cohort of healthy children and adolescents.

Methods: Approximately 200 healthy children and adolescents (5 to <19 years) were recruited from the community with informed consent. Whole blood was collected in a heparinized syringe and rapidly analysed at the point of collection for 11 parameters (i.e. pH, pCO2, pO2, COHb, MetHb, hemoglobin, potassium, sodium, chloride, ionized calcium, lactate) on the Radiometer ABL90 FLEX Plus platform. Reference intervals were then established in accordance with Clinical and Laboratory Standards Institute guidelines.

Results: All analytes, except hemoglobin, demonstrated very constant reference values across the pediatric age range, eliminating the need for stratification by age and/or sex. Hemoglobin required age- and sex-partitioning at the onset of puberty, with adolescent males demonstrating higher concentrations compared to females.

Conclusion: This study establishes accurate and robust reference standards for 11 critical care analytes in the healthy CALIPER cohort for the first time. These data greatly contribute to our understanding of normative pediatric values for electrolytes, metabolites and blood gases on a modern POCT instrument and can be expected to facilitate test interpretation in clinical institutions that use these assays.

A-307

Performance Evaluation of a Newly Developed Rapid Automated Fluorescent Lateral Flow Immunoassay for IgG and IgM Antibodies to Chikungunya Virus

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Background: Chikungunya virus (CHIKV) is a mosquito-borne virus belongs to the alphavirus genus of the family Togaviridae. The increase in overseas travel has led to the spread of viral infections worldwide, and the possibility of the outbreaks might be expected. We evaluated a newly developed rapid automated fluorescent lateral flow immunoassay to detect IgG and IgM antibodies to Chikungunya virus.

Methods: Ichroma CHIKV IgG/IgM (Boditech Med, South Korea) is a newly developed automated immunoassay for qualitative detection of anti-CHIKV IgM and IgG using a small bench-top fluorescence reader. We evaluated the diagnostic performance of Ichroma CHIKV IgG/IgM with Inbios CHIKjj Detect™ IgM Capture ELISA, Inbios CHIKjj Detect™ IgG ELISA (InBios International, USA) and Anti-Chikungunya virus ELISA (IgM), Anti-Chikungunya virus ELISA (IgG) (Euroimmun, Germany). Thirty six anti-CHIKV IgG positive sera and 57 anti-CHIKV IgM positive sera from CHIKV infection confirmed patients (purchased from Trina Bioreactives AG, Switzerland) and 163 anti-CHIKV IgG/ IgM negative sera from healthy Korean individuals were included.

Results: Both Euroimmun and Inbios ELISAs showed 100% sensitivity and 100% specificity for anti-CHIKV IgG and IgM detection. Ichroma detected all 36 anti-CHIKV IgG positive and 57 anti-CHIKV IgM (100% sensitivities). For 163 anti-CHIKV IgG/ IgM negative sera, Ichroma showed one false positive anti-CHIKV IgM result (99.4% (95% CI 97.5-99.4) specificity). Ichroma showed no cross-reactivity with other infections and no interference.

Conclusion: Ichroma CHIKV IgG/IgM demenstrated good diagnostic performance compared to the current ELISAs. The assay could be applied in clinical field for CHIKV serological diagnosis.

A-308

Analytical Performance of Point-of-Care Glucose Measurement in Hospitalized Adults and Children

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Background: POC blood glucose is typically determined in children and adults using capillary blood whereas laboratory glucose measurement typically uses venous samples. The study objective is to define the contributions of specimen type and method to the bias between glucose meters and laboratory measurement in hospitalized patients. **Methods:** Participants consented to simultaneous venous and capillary sampling. Glucose was measured immediately on aliquots of venous and capillary blood using 6 Abbott FreeStyle Precision Pro meters and 3 lots of reagent strips. Plasma from both capillary and venous blood was separated within 30 minutes of acquisition and glucose determined using the Roche Cobas 6000. 1,256 results were analyzed from 314 participants (55% male, age 1 day to 91 years, mean=44 years). 45 participants were less than 18 years-old and 42 were in an ICU. Venous plasma glucose was the gold standard. **Results:** Data are summarized in the Table. Venous Cobas glucose concentrations ranged from 42-449 mg/dL and meter-to-Cobas bias (mean±SD) was -0.1±11.8 and 3.8±15.7 mg/dL for venous (r^2=0.97) and capillary blood (r^2=0.95), respectively. Average bias between meter-capillary results and venous-Cobas results was 0.4±22.7 mg/dL. Correlation of glucose measurements between meter-capillary and venous-Cobas (r^2=0.89) was lower than venous meter-to-Cobas and capillary meter-to-Cobas (P<0.001, Fisher z-transformation). The distribution of bias for capillary-meter vs. venous-Cobas was greater in children (6.0±26.4 mg/dL) than adults (-0.6±21.9 mg/dL) and significantly larger in ICU populations (12±34.5 mg/dL) than in non-ICU populations (-1.5±19.6 mg/dL). **Conclusion:** The distribution of bias in glucose meters compared to laboratory glucose measurement is greater between heterogeneous sample types than between homogeneous sample types. This suggests that physiologic differences between capillary and venous blood are more responsible for bias in capillary glucose determination than the measurement system. **Funding:** Study funded by Abbott Diabetes Care.

	Bias vs. Venous, Cobas (mg/dL)	Bias (95% CI)	Bias, SD	Coefficient of Determination, r^2
All data (n=314)				
Venous, Meter	-0.1	-1.5,1.2	11.8	0.97
Capillary, Meter	0.4	-2.1,2.9	22.7	0.89
Capillary, Cobas	-3.4	-5.4,-1.3	18.5	0.93
Adults (n=269)				
Venous, Meter	-0.4	-1.8,1.0	11.8	0.96
Capillary, Meter	-0.6	-3.2,2.1	21.9	0.87
Capillary, Cobas	-3.5	-5.7,-1.3	18.1	0.92
Children (n=45)				
Venous, Meter	1.2	-2.4,4.9	12.1	0.98
Capillary, Meter	6.0	-1.9,14.0	26.4	0.93
Capillary, Cobas	-2.7	-9.1,3.6	21.1	0.95
ICU (n=43)				
Venous, Meter	4.1	0.0,8.1	13.2	0.96
Capillary, Meter	12.0	1.4,22.7	34.5	0.68
Capillary, Cobas	-0.5	-11.1,10.0	34.2	0.71

A-310

Improved Screening of Acylcarnitines and Amino Acids in Dried Blood Spots by LC-MS/MS

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Background: L-Carnitine (C0) assists CoA activated medium- and long-chain fatty acids in mitochondrial transport by forming fatty acylcarnitines, and are diagnostic markers for detecting both fatty acid oxidation and organic acid disorders. Currently, there are two widely used approaches in newborn screening for acylcarnitines and amino acids: derivatization prior to and without derivatization and flow injection MS/MS analysis of solvent extracted dried blood spots (DBS). However, these techniques suffer from partial hydrolysis of acylcarnitines during derivatization, time consuming sample preparation, and poor selectivity from matrix components which causes significant ion suppression and decreased signal sensitivity of the analytes. We present a novel, rapid, non-derivatized LC-MS/MS newborn screening method with improved separation of 22 acylcarnitines and 13 amino acids in DBS using a Raptor HILIC-Si 2.7 µm, 5 x 2.1 mm guard column with a total analysis time of 1.2 minutes.

Methods: 50 µL of whole blood was spotted on Whatman 903 neonatal protein saver cards to prepare a DBS card. The cards were dried for 1 hour at room temperature and a 3.0 mm disk (~3.0 µL whole blood) was punched out into a 2.0 mL Eppendorf tube. 200 µL of 85:15 acetonitrile: water containing known concentrations of stable isotope labeled internal standards was added to the tube, vortexed, and incubated for 20 minutes at room temperature on a microplate shaker with a speed of 400 rpm. The sample was then centrifuged for 10 minutes at 4000 rpm and 150 µL of the supernatant was filtered using a Thomson SINGLE StEP Nano Filter Vials prior to LC-MS/MS and flow injection MS/MS analysis.

Results: : 22 acylcarnitines and 13 amino acids present endogenously in whole blood and 16 internal standards were separated on a Raptor HILIC-Si guard column using 30 mM ammonium formate in water and acetonitrile mobile phases using gradient conditions at a flow rate of 0.6 mL/min with a total analysis time of 1.2 minutes including the re-equilibration. Detection was performed using ESI in positive ion mode using scheduled multiple reaction monitoring allowing for more than 7 points per peak.To demonstrate the magnitude of matrix interference and the improvement in data quality through the use of a guard column, DBS samples were extracted and analyzed first by flow injection MS/MS analysis at a 0.3 mL/min flow rate followed by the rapid LC-MS/MS method utilizing the guard column at 0.6 mL/min, and the signal intensities

of the two experiments were compared. The rapid LC-MS/MS method showed good retention, selectivity, and increased the signal intensities for acylcarnitines by 2- to 3-fold and amino acids by 10-fold.

Conclusion: A rapid LC-MS/MS newborn screening method has been developed for the analysis of both acylcarnitines and amino acids in dried blood spots for the detection of several inborn errors of metabolism. This method has been demonstrated to be as fast as flow injection analysis while both removing the need for derivatization and improving the data quality by leveraging the retention and selectivity afforded by the use of a guard column.

A-311

Quality Improvement in Glucose Reagent Labeling: Nursing and Point of Care Collaboration

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Background: A recent Joint Commission (JC) accreditation visit highlighted the need to improve quality control checks for Point of Care testing (POCT) reagents with revised expiration dates upon opening. Areas for improvement included testing locations without the appropriate opened and/or revised expiration date noted on glucose testing materials. The manufactures' recommendation states the glucose test strips expire 6 months after opening and the Quality Control (QC) reagents expire 3 months after opening. Without the proper date labeling of glucose reagents, the accuracy of patient results may be compromised if expired testing materials are inadvertently used. **Objective:** To implement process improvements in the current glucose reagent labeling workflow in order to eliminate labeling errors as well as expired reagents. **Method:** The Plan-Do-Study-Act (PDSA) model was employed to implement process changes and evaluate outcomes. Initial strategies included: Just-in-time training, re-education during monthly POCT audits, and publication of visual job aides. Initial education-based strategies did not elicit a significant increase in compliance. There was a need for a proactive process solution to eliminate labeling errors. A 6-month pilot program was instituted in 9 different hospital units, in which the participating units would obtain a monthly supply of pre-labeled glucose reagents from the POCT department. Each subsequent month the units would exchange any remaining supplies for new supplies appropriately labeled for the current month. The proposed workflow would ensure the glucose testing materials were used within the expiration date as well as eliminate labeling errors by the end user. At the completion of the 6-month pilot program, the proactive process solution was implemented throughout the Medical Center Campus. Implementation for the remaining units was staggered over a 6 month period adding approximately 10 units every other month. **Results:** Prior to the pilot launch, the participating units had an average compliance rating of 78.67%. Upon completion of the 6-month pilot program, average compliance increased to 98.99%, revealing a 26% increase in compliance ($p<0.001$). **Conclusions:** The Nursing and POCT teams worked in collaboration to develop a novel and attainable strategy to eliminate labeling errors for reagents with revised expiration dates. This proactive process solution enhances the quality of patient care received throughout the organization by ensuring reagents are used only within the manufacturer recommended expiration date.

A-312

Demonstration of Feasibility for Potassium Detection Using Point-of-Care Electrochemical Sensors with the Potential to Overcome Barriers for Optimal RAASi Therapy

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Background: Heart failure (HF) and chronic kidney disease (CKD) guidelines recommend maximal tolerable renin-angiotensin-aldosterone system inhibitor (RAASi) therapy. However, suboptimal dosage is commonly prescribed in clinical routine use since the therapy is associated with the risk of developing hyperkalaemia. At-home monitoring of potassium (K^+) will diagnose hyperkalaemia early and safeguard high dose RAASi therapy to improve patient outcome.

Methods: An enzyme-based assay for quantifying serum potassium utilizing electrochemical sensors designed for disposable dry reagents suitable for home use was developed. Performance evaluation was conducted using a potassium depleted serum matrix spiked across a dynamic range of 2 to 10 mM KCl, reflecting the intended clinical range for the device. Analytical performance was determined by comparison to the Ion-Selective Electrode (ISE) reference method at several concentration levels, as measured by both accuracy and precision of 20 replicates at each level. Additionally, interference from two monovalent cations, sodium and ammonium, was evaluated.

Results: Variability and the closeness of agreement between ISE and the electrochemical sensors were determined. The precision (CV%) across the three selected serum concentrations was 14.9% (2.9 mM), 11.2% (5.8 mM) and 9.9% (8.7 mM). The estimated correlation of determination (R^2) and the slope when regressing (linear) the electrochemical sensor on the reference method were 0.93 and 0.93, respectively. Utilizing the Student's t-test statistical analysis, the data showed that the mean of the low potassium control was statistically the same as the mean of the low potassium control with added sodium or ammonium, respectively, at 95% significance level.

Conclusion: The method provides accurate results as compared to the reference method with good precision and no observed interference. This demonstrates the ability of the assay to be further developed into a point-of-care assessment of potassium levels with limited interferences.

A-314

Comparison of Six Point of Care Blood Gas Analyzers including the Newly Developed iSmartCare 10

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Background: The results of point-of-care (POC) blood gas analyzers have an immediate impact on patient care. However, discrepant results between POC analyzers can confuse physicians and make proper treatment difficult. We aimed to compare five conventional blood gas analyzers and a newly developed POC blood gas analyzer, i-Smartcare 10 for the determination of ten items. **Methods:** The analyzers included are as follows: i-SENS i-Smartcare10, Radiometer ABL90 Flex Plus, Instrumentation Laboratory Gem Premier 5000, Abbott i-STAT, Nova Stat Profile pHOx Ultra and Siemens RapidLab 1265. Test items are: pH, pCO_2, pO_2, Na^+, K^+, Cl^-, iCa^{++}, glucose, lactate and hematocrit. Cl^- and lactate could not be evaluated in i-STAT. Method comparison was performed with de-identified residual whole blood samples (n=182~209) according to CLSI EP09-A3 guideline with RapidLab 1265 as a comparator. Two medical decision points (MDP) were selected to obtain the predicted MDP value and the 95% confidence interval (CI) calculated by Passing-Bablok regression. **Results:** The results of the mean difference (%) estimation are summarized in Table 1 (Bold numbers in Table 1 indicate a significant difference from preset allowable bias specifications). The Passing-Bablok analysis yielded correlation coefficients (*r*) ranging from 0.874 to 0.997. For most of the test items, the mean differences (%) were within acceptable bias limits and yielded acceptable bias at both MDPs. However, pCO_2, iCa^{++}, Cl^- and hematocrit in i-SmartCare 10, iCa^{++} in Gem Premier 5000, Na^+ and iCa^{++} in ABL90 Flex Plus, iCa^{++}, Cl^- and hematocrit in pHOX ultra, hematocrit in i-STAT showed significant mean differences (%) with significant bias at MDPs.Table 1.

	Mean difference (%)					Allowable bias, %
	i-Smartcare 10	Gem Premier 5000	ABL90 Flex Plus	pHOX ultra	i-STAT	
pH	0.41	0.41	0.28	0.00	-0.12	3.90%
pCO_2, mmHg	**-7.31**	-3.71	-5.05	-0.64	-0.81	5.70%
pO_2, mmHg	-0.82	7.11	7.22	-1.88	3.64	N/A
Na^+, mmol/L	-0.20	0.27	**-2.12**	-0.19	-0.31	0.73%
K^+, mmol/L	1.10	-0.45	0.83	-2.77	1.76	5.61%
iCa^{++}, mmol/L	**-4.72**	**-5.54**	**-3.73**	**-10.17**	-1.70	2.00%
Cl^-, mmol/L	**-2.18**	-0.77	0.91	**-3.18**	N/A	1.50%
Glucose, mg/dL	**-7.76**	-4.72	-3.83	-5.95	1.14	6.96%
Lactate, mmol/L	8.56	-1.92	0.61	2.13	N/A	30.40%
Hematocrit, %	**-10.94**	**5.82**	-3.20	**-9.47**	**8.43**	3.97%

Conclusion: Overall, five POC blood gas analyzers showed good comparability to Rapidlab 1265 with acceptable bias except for some items. This outcome suggests these blood gas analyzers could be useful in the clinical setting, but caution needs to be taken in interpreting the results of different analyzers.

A-315

Combined Assessment of Presepsin and Procalcitonin in Addition to the quickSOFA (qSOFA) Score Improve the Prediction of Mortality, Complicated Sepsis, and Septic Shock in Patients with Early Sepsis Admitted to the Emergency Department

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Background: A recently published comprehensive analysis of the incidence and mortality of sepsis has provided that incident cases and deaths are twice compared with previously known data (Lancet 2020;395:200-11), demonstrating that early and reliable diagnosis and prognostication of sepsis already ad admission is necessary. A new bedside clinical score was introduced in 2016 termed quickSOFA (qSOFA). The Third International Consensus Definitions for Sepsis and Septic Shock defined the qSOFA, which does not require laboratory tests and can be assessed at patient admission. Patients with worse outcome (e.g. death, major adverse events (MAE) or complicated sepsis) should be identified in case of qSOFA≥2 already at admission. But subsequent validation studies showed suboptimal overall discrimination and insufficient performance. The sepsis biomarkers presepsin (PSEP) and procalcitonin (PCT) have been shown to provide diagnostic and prognostic information in sepsis. In this study we thought to evaluate whether combining PSEP and PCT with qSOFA would improve its ability to predict poor outcome or mortality in septic patients already at admission.

Methods:

107 Patients admitted to the emergency department with signs of sepsis were included. Primary endpoint was death within 30 days. The combined endpoint "major adverse event" (MAE) consisted of at least one of the primary or the secondary endpoints need of intensive care, mechanical ventilation or dialysis. At first presentation EDTA plasma samples were collected. The qSOFA criteria respiratory rate≥22/min, altered mentation, or systolic blood pressure≤100 mmHg were documented. PSEP and PCT were determined using the PATHFAST instrument (LSI Medience Corporation, Japan) and the BRAHMS PCT luminescence immune assay (Thermo Fisher Scientific, Hennigsdorf, Germany).

Results:

Median values of PSEP and PCT were 688 (IQR: 391-1143) pg/ml, and 1.39 (IQR: 0.385-4.29) ng/ml in the group with qSOFA≤1 (N=76) and 1407 (IQR: 818-2267) pg/ml, p=0.0003, and 2.73 (IQR: 0.90-16.5) ng/ml in patients with qSOFA≥2 (N=31). The 30-day mortality was 12.1% (n=13) overall, in the group with qSOFA≤1 3.9 % (N=3), with qSOFA≥2 32.3% (n=10). The discrimination between survivors (n=95) and non-survivors (n=18) by ROC analysis revealed AUC values of 0.772, 0.519 and 0.802 of PSEP, PCT and qSOFA, respectively. Combination of PSEP, PCT and qSOFA by logistic regression revealed an AUC value of 0.850.

In patients with qSOFA≥2 PSEP≥1000 ng/L/PCT≥2 µg/L "rule in, Sensitivity" of death, MAE, and complicated sepsis revealed 100%, 72.2% and 75% compared to qSOFA alone with 32.3%, 58.1% and 64.5%, respectively.

In patients with qSOFA ≤1 the safety "rule out" of death, MAE and complicated sepsis could be improved compared to qSOFA alone by PSEP<1000 ng/L/ PCT<2 µg/L by 81.1%, 83.8% and 95.%, respectively.

Conclusion:

These findings show that the combined assessment of qSOFA, PSEP and PCT according to thresholds PSEP</≥1000 ng/L and PCT</≥2 µg/L improves the risk stratification of septic patients admitted to the emergency department significantly compared to qSOFA alone. PSEP and PCT with the PATHFAST system can be determined in parallel from anticoagulated whole blood immediately at admission within 17 minutes.

A-316

Evaluating the Torq™ Zero Delay CentrifugeSystem in At-Home Clinical Trial Patient Follow-Up Visits

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Background: Ensuring high quality specimens is a critical challenge in performing at-home clinical trial patient follow-up visits in which blood draws are conducted outside of a conventional draw center or laboratory. Our mobile healthcare professionals' (HEROs) existing options for prompt plasma stabilization have been limited to either transporting plasma separation tubes (PSTs) to a nearby laboratory, or using a portable, fixed angle centrifuge either in the patient's home or the phlebotomist's vehicle. Such limitations hinder the laboratory acceptance rate for assays susceptible to sample degradation (e.g. high hemolysis), lead to repeat patient visits for redraws, reduce the HERO's daily visit throughput, and constrain our geographic footprint for providing mobile patient follow-up services. The Torq™ Zero Delay Centrifuge System from Sandstone Diagnostics is a lightweight, hand-portable device for immediately separating and stabilizing plasma at the point of collection. The Torq system is comprised of the ZDrive™ - a 4" diameter, battery-powered centrifuge - and ZDiscs™ - evacuated and anticoagulated cartridges designed to collect whole blood and isolate liquid plasma from cells following a brief (4 minute) spin upon collection. The objective of this study was to evaluate the Torq System's usability by HEROs performing cardiology patient follow-up visits in ongoing trials measuring BNP and proBNP levels - two plasma-based analytes susceptible to whole blood degradation. A secondary objective was to assess difference in the centralized laboratory sample acceptance rate between Torq samples and PSTs. **Methods:** Torq ZDrives and 4mL EDTA ZDiscs were deployed to n= 7 HEROs, who used the device with n=21 patients participating in the clinical trials. HERO training included written instructions and a 5-minute online training video. During the visits, ZDiscs were loaded directly after draw via syringe and immediately spun in the ZDrive. Plasma was then extracted from the ZDisc by pipette and transferred to plasma transport tube for shipment to the centralized laboratory. HEROs were also asked to complete a brief usability survey assessing the system materials/supplies, training, and ease-of-use for each process step. **Results:** 7 out of 7 (100%) HEROs agreed that the Torq System performed as expected, and 7 out of 7 (100%) reported they liked using the Torq System. In addition, 21 out of 21 (100%) samples collected and processed with the Torq System were accepted by the laboratory. **Conclusions:** The Torq System provides a rapid, effective, and easy-to-use method for collecting and stabilizing plasma samples in at-home clinical trial at-home patient follow-up visits. Torq has been well-received by our mobile healthcare professionals, decreases our need for repeat patient visits, reduces our personnel's equipment burden, and has dramatically improved the laboratory specimen acceptance rate in pilot evaluation.

A-317

Aberrant Total Protein Findings in Cases of IgM Paraproteinemia Using a Point-of-Care Device

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IntroductionThe Piccolo Comprehensive Metabolic Profile (CMP), (Abaxis, Union City, CA) is a CLIA-waived method used as a point of care test (POCT) for metabolic profiling in our oncology clinic. Two patients were identified as having spuriously low total protein (TP) values using this POCT. Although paraproteins may elevate TP and cause interference in various laboratory assays, falsely low TP levels using the Piccolo POCT have not been previously reported.

MethodRelevant medical history and laboratory data for each patient were reviewed. Levels of CMP analytes from the POCT and Beckman Coulter DxC chemistry analyzer (DxC) were compared for the two patients. Additional cases of TP ≤ 6.0 g/dL on the POCT during this time period were reviewed.

Data The patients (A and B) with falsely low TP levels were determined to have IgM paraproteinemia (IgM plasma cell myeloma and Waldenström macroglobulinemia) with IgM levels of 2.24 g/dL and 6.99 g/dL, respectively. Comparing POCT to DxC, these patients had TP recoveries of 68.6% and 46.1% (See Table).

In contrast, none of the additional cases (C-H) with low TP values had an IgM paraproteinemia. For these patients, the average recovery was 102.4%.

Albumin measurements were also reviewed. Patients A and B had recoveries of 83.3% and 92.1%, respectively, compared to an average of 101% for Patients C-H. Albumin measurements appear less affected.

ConclusionFalsely low TP results were seen only in patients with IgM paraproteinemia, likely due to hyperviscosity associated with polymerization of pentameric IgM immunoglobulins. The falsely low TP values were not seen in other oncology patients, including those with IgA and IgG paraproteinemias. Serum hyperviscosity may have affected the accuracy and precision of POCT measurements by reducing the aspirated sample volumes and falsely decreasing concentrations of measurands. Confirmatory testing is advised for unexpectedly low TP measured by the POCT.

Piccolo POCT and DxC TP and Albumin Measurements						
Patient	Piccolo TP (g/dL)	DxC TP (g/dL)	Recovery TP (%)	Piccolo Albumin (g/dL)	DxC Albumin (g/dL)	Recovery Albumin (%)
A	4.8	7.0	68.6	2.0	2.4	83.3
B	4.7	10.2	46.1	3.5	3.8	92.1
C	5.8	5.9	98.3	3.0	3.4	88.2
D	5.5	5.7	96.5	3.2	3.6	88.9
E	5.8	6.1	95.1	3.1	3.2	96.9
F	4.1	3.9	105.1	1.5	1.3	115.4
G	4.6	4.4	104.5	2.7	2.5	108.0
H	5.7	5.6	101.8	3.2	3.5	91.4
I	5.9	5.1	115.7	3.3	2.8	117.9
Reference Interval	6.4-8.1	6.5-8.3		3.3-5.5	3.6-5.0	

A-318

Development of a Rapid, Quantitative, Multiplexed Point of Care Testing (xPOCT) for Procalcitonin (PCT), C-Reactive Protein (CRP), and Lactate (LAC) in Whole Blood

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Background: Point of Care Testing (PoCT) has been increasingly useful for assisting sepsis diagnosis since early screening and identification correlates directly to patient outcome/survival. Clinics commonly use the following measurements to facilitate the evaluation of sepsis: Procalcitonin (PCT), C-Reactive Protein (CRP), and Lactate (LAC). In particular, PCT measurement is useful to confirm or exclude the diagnosis of sepsis and guide antibiotic treatment if necessary. Currently, there is no FDA cleared IVD device for PCT measurements in whole blood sample type. Quantitative whole blood PoCT assay is much more desired for timely, on-site diagnosis of sepsis. We have developed a sensitive, multiplexed PoCT device for the detection of PCT, CRP, and Lactate in whole blood with built-in negative and positive controls. **Methods:** The whole blood PoCT device (WB-Panel) consists of an analyzer and a disposable assay cartridge, which contains antibodies and enzymes to generate signals from PCT, CRP, and Lactate. The experiment was run by loading 150μL of samples in the cartridge. The test was run on the analyzer, which simultaneously reported concentration values for all three assays at the conclusion of the run (~12.5 minutes). Both whole blood and plasma results were compared to those in the Nanomix eLab® S1 Panel, which is a CE Marked device for PCT, CRP, and Lactate in plasma. Preclinical study (n = 39) was also performed in University of California, San Francisco (UCSF) to establish a correlation between whole blood and plasma samples in the WB-Panel. **Results:** We noticed whole blood samples in the S1 Panel had 16% signal recovery, which would affect the lower end sensitivity and variability. We presented a newly developed device, WB-Panel, which successfully recovered the whole blood PCT signal by 70% (CV = 13.7%). The correlation between plasma and whole blood signals was determined to be highly linear (R^2=0.992) across the signal ranges tested (3nC-6000nC). We tested the dynamic range of all three assays: PCT (0.04-216 ng/mL), CRP (1-200 μg/mL), LAC (0.31-20mM). Negative and Positive Control signals remained constant. In a preliminary study, we confirmed that the WB-Panel signals were linearly traceable to the S1 Panel plasma signals for PCT, CRP, and LAC (R^2=1.00, 0.99, 0.94 respectively). Additionally, we compared whole blood to plasma signal correlations in clinical/preclinical samples for both panels. While CRP and LAC assays were not affected ($p>0.05$) in the WB-Panel, variability and signal recovery were improved in PCT assay. Also, the WB-Panel performed robustly across the hematocrit range from 23.8% to 51.3%. **Conclusion:** We have successfully developed a quantitative, rapid, multiplexed PoCT device for the detection of PCT, CRP, and Lactate in whole blood. The WB-Panel effectively recovered PCT signals in whole blood. Moreover, all three assays in the WB-Panel could be traced to plasma signals in the S1 Panel. From a preclinical study at UCSF, we identified that the WB-Panel did not affect CRP and LAC assay performance, but improved PCT assay performance comparing to the S1 Panel. All three assays showed wide dynamic ranges.

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Performance Characterization of Lactate Assay in a Rapid, Quantitative, Multiplexed Point of Care Device (xPOCT)

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Background: Sepsis, a body's response to an infection, is one of the main causes of mortality, and requires timely diagnosis and treatment for patient survival. Among many other measurements relevant for assisting sepsis management, lactate measurement has been useful for predicting mortality and initiating treatment. To facilitate the evaluation of sepsis, we developed a rapid, multiplexed, Point of Care Testing (PoCT) device that quantitatively measures Lactate (LAC), Procalcitonin (PCT), and C-reactive protein (CRP) levels in plasma. This report will specifically address the performance of the lactate assay in the multiplexed device.

Methods: The Nanomix eLab® S1 Panel, a CE Marked device, consists of an analyzer and a disposable assay cartridge, which contains antibodies and enzymes to generate signals from PCT, CRP, and Lactate. Each cartridge utilizes a biosensor that detects electrochemical signals in nano-coulombs (nC) generated from a lactate enzymatic reaction. The experiment was run by loading 150 μL of samples in the cartridge. The test was run on the analyzer, which simultaneously reported concentration values for all three assays at the conclusion of the run (~12 minutes). Analytical performance such as interference, precision, detection limits, and linearity was tested per CLSI guidelines. Analytical accuracy and matrix effect were also investigated and compared to the i-STAT® CG4+ LAC test (Abbott, USA). Only lactate data is summarized and presented in this poster.

Results: We determined the linear range of the lactate assay to be from 0.2083mM to 15mM. We established the Limit of Blank (LoB=0.012mM), Limit of Detection (LoD=0.035), Lower Limit of Quantification (LLoQ=0.3125mM), and Upper Limit of Quantification (ULoQ=7.5mM) according to CLSI guidelines. Combining the above results, the lactate reportable range was determined to be from 0.3mM to 7.5mM. To test the precision, we conducted three studies testing site/operator variability (n=268), lot/instrument variability (n=180), and day-to-day variability (n=80). The total variability for all samples tested was less than a CV of 15%. Among forty substances tested for interference, oxaloacetic acid at 112.5mg/dL was found to decrease the lactate concentration, and glycolic acid (4.5mM) had an additive effect with oxaloacetic acid (59.4mg/dL) to decrease the lactate concentration. In a comparability study between i-STAT and S1 Panel using a certified reference material diluted in different sample matrices, we found that the S1 Panel did not experience any matrix effect, and reported concentration values closer to the reference lactate material than did the i-STAT. Moreover, when the reference material was spiked with high concentrations of CRP, the i-STAT was found to underreport lactate concentration, while the S1 Panel remained unaffected.

Conclusion: We successfully developed and characterized the lactate assay in a multiplexed PoCT device, Nanomix eLab® S1 Panel. The reportable range was determined to be from 0.3mM to 7.5mM. The assay precision was 15% CV for all the conditions tested. Two substances, oxaloacetic acid, and glycolic acid contributed to the lower reading of lactate concentration. The S1 panel lactate assay was found to have no obvious matrix effect, and it reported accurate measurements of lactate concentration as compared to the i-STAT device.

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Validation of an ELISA and a POC Device for Measuring Synovial Calprotectin with Correction for Blood Contamination

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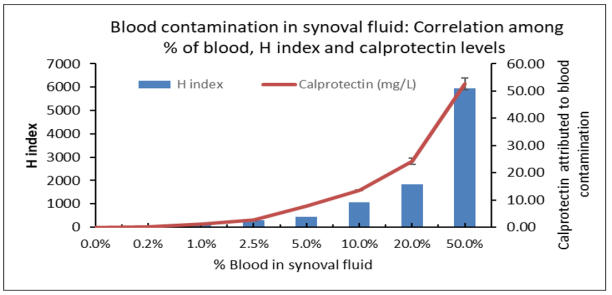
Background: Synovial calprotectin is a novel biomarker for prosthetic joint infection (PJI). We evaluated a Point of Care (POC) and an ELISA test (Lyfstone, Norway) for detecting calprotectin in synovial fluid (SF). Given the propensity of blood contamination during SF collection, we also investigated the interference of blood contamination and developed correction methods. **Method:** The ELISA was performed using the lab-developed protocol on a DSX analyzer. The POC test was performed per manufacturer's instruction. Hemolysis (H) index of SF was measured using a c501 analyzer (Roche, IN). SF was obtained intraoperatively from consented revision total knee arthroplasty patients of an IRB approved study. The reference interval of synovial calprotectin was established using SF from primary total knee arthroplasty patients by the ELISA. Blood interference studies consisted of mixing whole blood and SF at various ratios and measuring calprotectin and H index. **Results:** The performance characteristics were summarized in Figure 1. The POC test was reproducible

for determination of low, moderate or high risk for PJI using manufacturer cutoff values, but showed higher imprecision and lower sensitivity in measuring calprotectin concentration than the ELISA. H index in combination with a polynomial regression model showed 99.1±8.4% recovery in estimating % blood in SF. About 48% (36/75) SF samples showed contamination of >1% blood which was equivalent to >0.8 mg/L calprotectin. About 19% of samples were shown to contain ≥10% blood which could lead to false-positive POC results. Synovial calprotectin levels were corrected for blood contamination using either average or individual blood calprotectin concentration with % blood in SF calculated from H index. 139±55% and 102±20% correction was achieved when using the average and individual blood calprotectin respectively. **Conclusion:** The study characterized the ELISA and the POC assay for detecting synovial calprotectin and established a method for eliminating interference of blood contamination.

Figure 1	**Synovial Calprotectin ELISA**		**Synovial Calprotectin POC**	
	Concentration (mg/dL)	Intra-/Inter-assay CV	Concentration (mg/L)	Intra-/Inter-assay CV
Low synovial fluid pool	1.04	4.9%/13%	41.6/21.6	14.4%/52.5%
High synovial fluid pool	78.1	5.4%/5.3%	275.2/67.5	7.6%/25.5%
Spike Recovery	0.5 mg/L 50 mg/L	77-89% 96-100%		
AMR Reportable range	0.08-5.0 mg/L 0.08-250 mg/L		Qualitative: low, moderate and high; 14-300mg/L	
Reference Interval	<0.8 mg/L		Low risk:<14mg/L; Moderate: <30 mg/L; High risk: >30 mg/L	
Method Comparison X: ELISA; Y: POC (n=73)		R: 0.9120 Bias: -3.7mg/L	Slope: 0.60 Intercept: -1.05	

Correlation: H index vs %blood r=0.996; H index vs Calprotectin r=0.986



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Diagnostic Utility of a Novel Point-of-Care Test of Calprotectin for Periprosthetic Joint Infection after Total Knee Arthroplasty

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Background: Despite several synovial fluid biomarkers for diagnosis of periprosthetic joint infection (PJI) being investigated, point-of-care (POC) tests using these biomarkers are not widely available. Synovial calprotectin has recently been reported to effectively exclude diagnosis of PJI and a novel lateral flow POC test has shown potential to be effective. Thus, the objective of this study was to test the sensitivity, specificity, positive, and negative predicted values (PPV and NPV, respectively) of a calprotectin POC test for PJI diagnosis in total knee arthroplasty (TKA) patients using the 2013 Musculoskeletal Infection Society (MSIS) PJI diagnosis criteria as the gold standard. **Methods:** Synovial fluid samples were prospectively collected from 120 patients that underwent revision TKAs (rTKA) at two academic hospitals within the same institution from October 2018 to December 2019. The study was conducted under IRB approval. Included patients followed the hospital standard for their PJI diagnostic work-up, and data collection comprised demographic, clinical, and laboratory data in compliance with MSIS criteria. Synovial fluid samples were analyzed on a calprotectin POC test (Lyfstone, Lysaker, Norway) in accordance with manufacturer's instructions. Quantitative calprotectin is reported by the test system as either high risk (>50 mg/L), medium risk (14-50 mg/L) or low risk (<14 mg/L) for infection. Patients were categorized as septic or aseptic using MSIS criteria by two independent reviewers blinded to the calprotectin results. Calprotectin test performance characteristics with sensitivities, specificities, PPV, NPV, and areas under the curve (AUC) were calculated for 2 different PJI diagnosis scenarios: (1) a threshold of >50 mg/L and (2) a threshold of >14 mg/L. **Results:** According to MSIS criteria, 53 rTKAs were septic while 67 rTKA were aseptic. The corresponding calprotectin classification read-outs of these cases were 52 high risk and only 1 low risk for the MSIS criteria septic cases and 58 low risk, 6 medium risk, and 3 high risk for the MSIS criteria aseptic cases. In the (1) >50 mg/mL threshold scenario, the calprotectin POC performance showed a sensitivity, specificity, PPV, NPV, and AUC, respectively, of 98.1%, 95.5%, 94.5%, 98.5%, and 0.968. In the (2) >14 mg/mL threshold scenario, the sensitivity, specificity, PPV, NPV and AUC were 98.1%, 86.6%, 85.2%, 98.3%, and 0.923, respectively. **Conclusion**: The calprotectin POC test has excellent PJI diagnostic characteristics including high sensitivity and specificity in rTKA patients. This test could be effectively implemented as a rule out test. However, further investigations with larger cohorts are necessary to validate these results.

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Significant Improvement of Lactate Turnaround Time (TAT) for Sepsis Diagnosis using a Laboratory Instrument in a Point-of-Care (POC) Setting

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Background: Sepsis is defined as a dysregulated response to an infection that leads to life-threatening organ dysfunction. The diagnosis of sepsis relies on assessment of multiple factors, including clinical signs and symptoms, laboratory results and radiologic studies. In 2015, we implemented a standardized sepsis protocol based on Surviving Sepsis Campaign guidelines, which included a Sepsis Best Practice Alert (BPA) that applies the Systemic Inflammatory Response Syndrome (SIRS) criteria to alert providers to order a lactate level when at least TWO of the following criterion are met within the last 24 hours:• WBC > 12 or < 4 x 10^9/L • Systolic BP < 90 mmHg • Temp < 95 or > 101°F • Respiratory Rate > 20• Heart Rate > 100 bpm The goal for lactate TAT was ≤60 min from alert to result, based on Surviving Sepsis campaign guidelines. After implementation of the BPA it was determined that lactate results were often delayed, with only 50 - 58% resulted in ≤60 min. Assessment of workflows and processes determined that the delay was primarily due to the time needed for transport and testing in the centralized core laboratory. Therefore, we conducted pilot studies on three patient units to determine whether POC lactate testing would adequately improve lactate TATs.

Methods: Three Radiometer ABL90 analyzers were configured to run lactates only at POC on three critical patient care units. To facilitate the prompt documentation of results, a wireless communication interface sent results directly to the patient's electronic medical record. Approximately 55 front-line laboratory staff were trained to perform the testing. Data management included the use of Epic and Telcor software to assess instrument and lab staff performance and compliance. This process allowed metric analysts to evaluate project results, identify issues, and implement countermeasures to ensure the accomplishment of goals.

Results: Over the course of all three pilot studies, laboratory staff collected, analyzed and documented approximately 6900 specimens on assigned units. TATs were calculated using data generated from three different sources: Telcor, Epic, and documentation logs. TAT data and personnel compliance evaluations were performed on a weekly basis. Lactate TAT compliance improved from 50 - 58% in ≤60 min to 75 - 90% in ≤60 min.

Conclusion: Placement of three ABL90 analyzers at the point-of-care significantly improved the capability of the laboratory to provide rapid TATs, to meet the goals of the sepsis protocol. Improved lactate TATs provide physicians and nurses key information required to rapidly assess the patient's condition, minimizing the time to treatment initiation which improves patient morbidity and mortality.**Keywords:** Lactate, Sepsis Protocol, Point-of-Care

A-324

Method Comparison of a New QuikRead Go HbA1c Test to Three Commercial POC HbA1c Tests and to IFCC Calibrated Reference Method

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BACKGROUND: QuikRead go HbA1c is an immunological in vitro diagnostic test for quantitative measurement of glycated haemoglobin (HbA1c) from finger prick or anticoagulated venous whole blood. The test is carried out using the QuikRead go

instrument. Aim of the study was to compare the upcoming QuikRead go HbA1c test to IFCC calibrated (SRMP) reference method and to three commercial Point-of-Care (POC) HbA1c CE marked tests already available on the market.

METHODS: Method comparison of QuikRead go HbA1c test (y) to three POC assays (x) was performed by measuring 78 venous whole blood samples as singlicates. Testing was performed according to CLSI EP09c-3rd edition. Samples were also analyzed with IFCC calibrated HbA1c TosohG8 HPLC reference method. Weighted Deming regression analysis was used for the data analysis.

RESULTS: QuikRead go HbA1c test (y) demonstrated an excellent correlation to a boronate affinity POC method (x) y=1.02x-0.2 (POC A), and to two immunological POC methods (x) y=1.03x-0.9 (POC B) and y=1.05-1.3 (POC C). All tests had an excellent correlation with r=0.99. When these four methods were compared to the IFCC calibrated (SRMP) TosohG8 HPLC reference method (x), following results were obtained: QuikRead go HbA1c (y) y=1.03x-0.9, POC A (x) y=1.01x-0.8, POC B (x) y=1.04x-4.2 and POC C (x) y=0.98x-0.3. The correlation coefficient to Tosoh G8 was high for all of the tested POC tests (r=0.99).

CONCLUSIONS: The obtained results indicate that QuikRead go HbA1c is very well in line with the HbA1c reference method and with tested POC methods. QuikRead go HbA1c has proven to be a reliable and effective method for the quantitative determination of HbA1c.

A-325

Discovery of the New Starch Reaction and Determination of the Effect of Pralidoxime on Blood Glucose Measurement

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Background: Pralidoxime (PAM iodide, PAM-chloride) serves as an antidote to organophosphorus (OP) pesticide poisoning by reactivating acetylcholinesterase and cholinesterase. There has been an increase in the number of suicides by OP pesticides in farm village areas in Asia owing to increases in financial problems, destitution, and anxiety. Recently, the number of patients with diabetes has increased rapidly worldwide; therefore, controlling blood glucose (GLU) levels is important for health care. The widely used methods for GLU determination are self-monitoring of blood glucose (SMBG) and flash glucose monitoring (FGM). However, PAM may influence the outcome of clinical laboratory tests based on these assays. Objective: To develop a new iodine-starch reaction-based method using PAM iodide and investigate the effect of PAM on GLU by SMBG.

Methods: The effect of PAM iodide on GLU was examined using three SMBG meters (SMBG1: Free Style Libre (FGM), SMBG2: Glutestmint, SMBG3: Glutest Every) (N=10). Various PAM iodide concentrations (PAM final concentration: 0, 0.19, 0.38, 0.75, 1.5, 2.25, and 3.0 mmol/l) were prepared. Then, 100 µl of 100 mg/dl glucose solution and 100 µl of PAM iodide solution were added to 800 µl of whole blood plasma samples, respectively. In the glucose oxidase (GOD) electrode method, GOD reacts with glucose and produces hydrogen peroxide (H_2O_2). We hypothesized that iodide (I^-), which is present in the molecular structure of PAM, was altered to triiodide (I_3^-) by H_2O_2; this was proved via a new iodine starch reaction. 500µl of PAM solutions (final concentration: 1.5, 3.0, 6.0 mmol/l) were added to 500µl of 1% H_2O_2, followed by the addition of 4ml starch solution. The absorbance of the resultant blue-colored solution was measured at 600 nm (N=7).

Results: The GLU levels after the addition of PAM iodide solution were as follows (mean ±SD): SMBG1, 117±4.3 mg/dl; SMBG2, 113 ±2.4 mg/dL; and SMBG3, 166 ±3.7 mg/dl. It became unstable and could not be measured in PAM solutions with concentration more than 3.75 mmol/l by SMBG1 and SMBG2 (GDH: glucose dehydrogenase method), because the effect of iodide (I^-) present in PAM was suggested for the electrode. In SMBG3 (GOD method), GLU levels increased proportionately with increase in PAM concentration from 108 mg/dl to 227 mg/dl (n=10, $p<0.01$). Similarly, GLU levels increased only PAM solution. The triiodide was confirmed using the new iodine starch reaction. The addition of starch solution to PAM resulted in a blue-colored solution; the absorbance of the blue solution significantly and proportionately increased with increasing PAM concentrations ($p<0.01$). In the conventional starch reaction, starch reacts with iodine (I_2) and KI solution. The PAM iodide solution is colorless and is safe for the environment.

Conclusion: PAM iodide had an influence on GLU measurement by SMBG (electrode method). Furthermore, using a response of PAM iodide, we discovered the iodine starch reaction newly for the first time. This study suggests that new starch reaction (PAM+ H_2O_2) may be useful to detection for iodide, starch and amylase enzyme.

A-326

Is the Quantra® QPlus® System Easy to Interpret?

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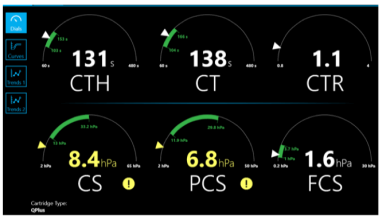
Background: Management of acute bleeding requires timely assessment of a patient's coagulation status. Viscoelastic testing (VET) combined with goal-directed algorithms has proven to be effective in improving patient care while minimizing overtreatment. However, the uptake of conventional VET methods into clinical practice has been limited, at least in part, due to the difficulty of interpreting results from existing devices. The Quantra QPlus System, a new cartridge-based VET device, offers a fundamentally different approach that uses an intuitive dials display (see Figure). The system outputs six parameters that relay information regarding the functional status of a patient's coagulation system. The objective of this study was to assess the ability of potential users to correctly interpret results displayed on the Quantra analyzer dials display screen.

Methods: Following a short training session, participants were presented with a case study booklet containing multiple Quantra display screens. The booklet contained multiple choice questions regarding specific interpretation of the information generated by each dial as well as clinical interpretation of the patient's status (hypocoagulable, hypercoagulable, normal, etc.) Study participants included 14 users from 3 anesthesia departments across the US. Participants represented a range of years of experience, all were board certified in one or more areas of anesthesia, and most had at least some experience with VET.

Results: For all the QPlus parameters, >95% of questions pertaining to each Quantra dials display and clinical interpretation were answered correctly.

Conclusion: This study demonstrated that with minimal training, clinicians presented with the Quantra results output for the first time were able to correctly interpret results displayed on the dials display screen. This suggests that one of the barriers limiting the widespread adoption of point-of-care viscoelastic testing may be overcome by the simple intuitive design of the Quantra System results output.



CTH: Heparinase Clot Time CT: Clot Time
CTR: Clot Time Ratio
CS: Clot Stiffness
PCS: Platelet Contribution to CS
FCS: Fibrinogen Contribution to CS

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The Impact of Anticoagulants on Glucose Measurement by a Point of Care Device

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Background: Point of care glucose testing in neonatal units is widely practice. However, lack of concordance with laboratory-based glucose values are often a concern. Samples sent to the laboratory for confirmation testing are collected into blood tubes containing recommended anticoagulants. This study examined impact of common anticoagulants, like lithium heparin, and chelating agents, like K-EDTA and Na-citrate on the measurement of whole blood glucose when using a point of care device.

Methods: Capillary blood samples collected from heel puncture were initially analyzed by Nova Glucose meter (StatStrip, Nova biomedical, MA) point of care instrument and a blood sample placed into a lithium heparin tube was prepared and sent to the laboratory for glucose measurement (Cobas, Roche Diagnostics, IA). The impact of anticoagulant on POCT glucose measurement was assessed by spiking glucose-depleted whole blood samples with glucose concentrations ranging from 30 to 300 mg/dL in the presence of the anticoagulants Na-citrate, lithium heparin and K-EDTA. Data obtained were analyzed using EP Evaluator software.

Results: Glucose level ranged from 10 to 101mg/dL when measured by both POCT and laboratory-based assay with an overall negative bias of -7.8 mg/dL. However, in the presence of lithium heparin anticoagulant and analysis by both methods, the overall median bias was lower at -1.0 mg/dL. Glucose values less than 40mg/dL showed lower negative bias when placed into a lithium heparin tube and measured by POCT (-8 to 13, median: -1 mg/dL) compared to direct measurement (-15 to 11, median: -4 mg/dL), both compared to laboratory-based glucose measurements. The presence of

anticoagulants tested caused positive bias in glucose values (-4 to 123, median: 6.8 mg/dL0, 22 to 276, median: 88.8 mg/dL), and (15 to 193, median: 52.0 mg/dL) when using Na-citrate, lithium heparin, and K-EDT anticoagulants respectively.

Conclusion: Acceptable concordance was observed for capillary blood glucose levels measured using the Nova blood glucose meter and Cobas- laboratory based methodology. The presence of anticoagulants caused marked positive bias in POCT glucose results. The use of anticoagulants should be avoided.

A-329

Unintended Consequence of High-Dose Vitamin C Therapy: Evaluation of Ascorbic Acid Interference with Three Glucose Monitoring Devices

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Background: The use of high-dose vitamin C as an adjuvant therapy in the treatment of cancer has shown promising results for some oncology patients. However, the intravenous (IV) doses used for these patients can reach concentrations that interfere with some glucose meter devices. We recently observed a case of repeat invalid glucose results in a patient who had received high doses of IV ascorbic acid for the treatment of recurrent lymphoma. The aim of our study was to investigate the impact of interference at varying concentrations of vitamin C on all three FDA-approved hospital use glucose meters. **Methods:** A working solution of ascorbic acid (25 mg/mL) was prepared from Ascorbic Acid Injection, USP [500 mg/mL] (Mylan, Canonsburg, PA) and 0.9% saline. Three pools of residual waste lithium heparinized whole blood were collected with low, normal, and high glucose concentrations. Aliquots from each pool were spiked with varying ascorbic acid concentrations. An equal volume of saline (<10% dilution) was added to control samples to serve as a reference. Each spiked sample from the three glucose concentration pools were measured in duplicate on the following FDA-approved hospital use glucose meter technologies: meter 1—Nova StatStrip Glucose Hospital Meter System (Nova Biomedical, Waltham, MA); meter 2—Roche Accu-Chek Inform II System (Roche Diagnostics, Indianapolis, IN); and meter 3— Abbott Precision Xceed Pro (Abbott Laboratories, Abbott Park, IL). Duplicate measurements were averaged, and the glucose difference was calculated using the $mean_{spike}$ and $mean_{control}$ values. **Results:** Ascorbic acid interference was assessed on all 3 commercially available hospital use glucose meters at ascorbic acid concentrations from 0-200 mg/dL. Across all glucose concentrations tested (30, 74, and 249 mg/dL), increasing concentrations of ascorbic acid resulted in a positive bias for both meters 2 and 3. The extent of interference was greatest with the low glucose concentration pool (>13-fold increase for meter 2 and >15-fold increase for meter 3). However, interfering concentrations on meter 1 resulted in instrument "errors" rather than inaccurate meter results. **Conclusion:** While typical doses of vitamin C should not cause interference with glucose meter testing, large doses administered in the treatment of cancer patients may result in significantly elevated ascorbic acid concentrations that impact blood glucose measurements. The Nova StatStrip glucose meter effectively detected the presence of ascorbic acid interferant, however glucose results were suppressed. Meanwhile, the Roche Inform II and Abbott Precision Xceed Pro demonstrated falsely increased results. It is important that providers are mindful of the differences between various glucose meter technologies and their susceptibility to interfering substances so that care is not negatively impacted and patients do not receive inappropriate treatment.

A-330

An Optical Waveguide Platform for the Rapid, Wash-Free Detection using Microsamples: Detection of PCT in Spiked Plasma

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Background: There is an unmet need for high quality and easy to use diagnostic tests in decentralized care settings. We here present the development of a disposable silicon-based sensor chip that allows the user to perform a bioanalytical assay, that is accurate and clinically relevant and provides a result in 10 minutes or less using a minimal sample volume (e.g. a finger prick of blood). This platform leverages the manufacturing infrastructure of the semiconductor industry, which is easily scalable to large production volumes thus reducing cost. The working principle is based on using on-chip photonic waveguides for the evanescent, fluorescent excitation and detection. This allows sensing only surface-bound labels without the unbound labels in the bulk enabling elimination of wash steps usually needed in sandwich immunoassays and perform wash-free assays with kinetic readout. The small footprint of the waveguides, combined with on-chip routing and multiplexing, enables large scale parallelization. We demonstrate the usefulness of the platform with the rapid, wash-free, detection of Procalcitonin (PCT) - a marker that can help in discriminating between bacterial and viral infection- at sub-picomolar concentrations directly in plasma. **Methods:** Single mode silicon nitride (SiN) waveguides, designed for 638 nm excitation light and each occupying 400 µm x 150 µm, were fabricated using standard silicon processing technologies. The waveguide surface was coated with an azidoalkylsilane monolayer via vapor-phase deposition to covalently link capture antibodies using cupper free click chemistry. For each measurement, 10 µl of fresh PCT-spiked EDTA-plasma (from healthy donors) was mixed with 30 µl of buffer with fluorophore labelled detector antibody. The signal buildup of the samples was monitored kinetically by measuring the increase of fluorescent light coupled out of the chip to an sCMOS imager. The slope of the fluorescent signal buildup between 60 and 120 seconds after sample application was used as readout. **Results:** The SiN waveguides demonstrated low propagation losses of excitation light (1.2 dB per cm) allowing ~ 0.1 mW of optical power inside the optical system (easily available from a small, disposable laser diode) to be split over 128 individual sensing waveguides and routed to a single output coupler array for highly parallelized measurements. Optimization experiments performed in buffer solutions showed the platform allowed kinetic detection of fluorescent signal buildup while rejecting bulk background by unbound detector antibody. For the final detection of PCT directly in spiked plasma samples, the average of triplicate measurements per PCT dose resulted in linear dose-response ($R^2 > 0.97$) for 0.013 - 1.65 ng/ml PCT in plasma (with clinical cut offs of 0.1 and 0.5 ng/ml to distinguish viral from bacterial infection in case of sepsis), corresponding 250 fM - 32 pM after dilution in assay buffer. **Conclusion:** We introduce here a mass manufacturable platform that allows the rapid miniaturized detection of biomarkers in small sample volumes. We demonstrate its ability to quantitatively detect relevant concentrations of biomarkers present at low concentrations such as PCT. This is an important step towards low-cost diagnostic tool that can be used in a decentralized setting. **Acknowledgements:** We acknowledge financial support from miDiagnostics.

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Towards the Point-of-Care Quantitation of Midregional Pro-Adrenomedullin through Sensitive Electrochemical Proximity Assay

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Background: Andrenomedullin (ADM) is a 185-amino-acid hormone expressed in many tissues and organ systems including cardiovascular, renal, pulmonary, cerebrovascular, gastrointestinal and endocrine tissues. It is a circulating hormone with autocrine and paracrine functions. The plasma concentration of ADM is elevated in patients suffering from heart failure, sepsis and acute myocardial infarction (AMI). The unstable nature of ADM *in vitro* and *in vivo* poses a challenge to using it as a biomarker. This issue has been overcome by measuring the midregional fragment of pro-ADM (MR-pro-ADM, amino-acid 45 to 92 of ADM), which is stable and in equimolar concentrations to its precursor. Recent studies have demonstrated the prognostic potential of MR-pro-ADM as a biomarker in acute heart failure (HF). Frequent at-home measurements of MR-pro-ADM by patients who are diagnosed with HF may be useful for monitoring health, but currently there are no point-of-care (PoC) assays available for this biomarker. InnaMed, Inc. is developing an affordable, at-home, connected diagnostic system, and is focused on quantification of MR-pro-ADM for heart failure patients using the patented electrochemical proximity assay.

Methods: The electrochemical proximity assay (ECPA), uses a dual antibody/aptamer sandwich complex formation for sensitive and selective electrochemical protein quantification. Insulin and thrombin were successfully quantified with LODs of 100 fM and 50 pM, respectively. First, a thiolated DNA is immobilized on the surface of a gold electrode. Next, the primary antibody (AB1) conjugated to DNA is introduced. This DNA hybridizes with the thiolated-DNA, strongly covering the surface of the electrode with AB1. The secondary antibody (AB2), also conjugated with DNA is strongly hybridized with a redox-molecule conjugated DNA (5MB-DNA). The MR-proADM sample is briefly mixed with the AB2-5MB-DNA complex and then dropped on the AB1-modified electrode. When the antibodies construct a sandwich on the surface in the presence of MR-proADM, the 5MB-DNA hybridizes with the thiolated-DNA (due to a proximity effect), placing the 5MB-DNA closer to the electrode surface. This 5MB-DNA is electrochemically measured using square-wave voltammetry and the current is proportional to the target concentration. This assay can be done in

a mix-and-read workflow (no wash steps or additional substrate are required), and is suitable for automated microfluidic PoC or at-home measurements, eliminating the need for experienced technicians.

Results: Our preliminary data shows successful detection of MR-pro-ADM with an LOD of 5 nM, as well as an assay time of less than 45 minutes. In addition, this assay demonstrated a qualitative distinction between healthy and sepsis patient serum samples.

Conclusion:

Preliminary measurements of MR-pro-ADM using ECPA were completed. These results are promising for further development of a sensitive assay. A PoC mix-and-read measurement system with a short turn-around time (<10 minutes) is being developed by improving upon this ECPA system and using InnaMed's automated fluidics (in development). The development of such an at-home, connected diagnostic device suitable for frequent monitoring of MR-pro-ADM can be supportive in management of patients with HF.

A-335

Clinical Validation of Point-of-Care Parathyroid Hormone Biosensor

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Background: Parathyroid Hormone (PTH) measurement is essential for the assessment and management of patients with parathyroid gland dysfunction with hyperparathyroidism, being the third most common endocrine disorder. In addition to diagnosis and management, PTH measurement is essential to guide surgical interventions where due to its short half-life (1-3 minutes) an intraoperative decline in circulating levels indicates successful surgical resection of a hyperfunctioning adenoma. Laboratory-based assays at best take up to 30 minutes to deliver results from sample receipt into the laboratory. In patients with failed initial surgery, a resection surgery several weeks later which is repeated once laboratory PTH data is available. This highlights the need for a rapid and reliable PTH assay that facilitates point of surgery testing (POST). Previously we have demonstrated rapid and reliable PTH detection in undiluted human serum, plasma and whole blood using Rapid Electro-Analytical Device (READ) platform. READ is a POST device that uses impedance change between target and capture probe to assess the target PTH concentration in undiluted patient samples. This work focuses on the clinical validation of the developed platform to be utilized as a clinical utility as a guide to surgeons. **Methods:** The sensor response for the targeted PTH analyte in plasma was evaluated using electrochemical impedance spectroscopy (EIS) over a spectrum of frequency. Cross reactivity of the sensor was tested against adrenocorticotropic hormone (ACTH), parathyroid hormone-like related protein (PTHrp) and cortisol. Leftover, de-identified discard samples (heparinized plasma) (n=40) were obtained from Clements University Hospital (CUH) and Parkland Memorial Hospital (PMH) from patients being investigated for thyroid dysfunction and undergoing parathyroid surgery. Key performance tests included sensitivity, linearity, precision, and interference were evaluated with 40 patient samples. Correlation and Balnd Altman comparison was evaluated between READ and reference standard analyzer (Roche diagnostic Cobas analyzer). **Results:** Quantification of PTH concentration in plasma samples was assessed using change in impedance observed over a frequency range using EIS. The intra-assay and inter-assay precision (%CV) of READ was 3% and 8 % for low level (38.2 pg/mL) sample whereas intra assay & inter-assay precision (%CV) for high level (942 pg/mL) sample was 3% and 10% respectively. The functional sensitivity within 15% coefficient variation was 1.9 pg/mL. Linear regression had a slope of 0.96 with Pearson's r=0.99. Mean bias between Roche analyzer and READ platform was 7.6 pg/mL with ±1.96 SD assessed using residual analysis such as Bland Altman. READ showed no cross-reactivity with PTHrp nor ACTH, or cortisol. READ platform shows ~1 pg/mL as a detection limit with a clinically relevant range of detection range from 1 pg/mL to 1 ng/mL. **Conclusion:** Developed READ platform demonstrates rapid results, a feature that is important when being used intraoperatively without sample dilution thereby, reducing sample processing and handling time. The wide dynamic range detection capability of READ to report significantly elevated PTH levels seen in patients suspected of hyperparathyroidism can be categorized as candidates for parathyroid surgery. READ also showed good precision across the measurement range and has good accuracy performance that helps to capture the low PTH levels post-surgical excision.

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Comparative Analyses of 3 Point-of-Care Urine Drug Screens Versus Mass Spectrometry

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Background: Urine drug screening is clinically useful in assessing patient (non)adherence/misuse of prescribed or illicit drugs in emergent and pain management settings. Point-of-care (POC) testing may be used for rapid urine drug screening, and is frequently requested in ambulatory settings void of on-site laboratories at our institution. This study aims to evaluate the performance characteristics of 3 different POC urine drug screening devices. **Methods:** 106 residual urine samples were used to evaluate 3 POC urine drug screen devices, where presence of drugs was determined by mass spectrometry during routine clinical testing. Urine samples were selected to challenge assay cutoff concentrations and cross-reactivity spanning 6 drug classes including: amphetamines (AMP), barbiturates (BAR), benzodiazepines (BZO), buprenorphine (BUP), cocaine (COC), methamphetamines (MAMP), methadone (MTD), opiates (OPI), oxycodone (OXY) and cannabinoids (THC). Samples were blindly tested by one laboratory professional (PhD) on the Profile®-V MEDTOX Scan® drugs of abuse test system with autoreader, Quidel Triage® TOX Drug screen with autoreader, and Quidel Triage® Rapid OXY-BUP-MDMA panel by visual interpretation. Sensitivity and specificity for drug classes/compounds were calculated for each POC device compared to the reference method. **Results:** 82 samples were positive for 1 or more drugs as determined by quantitative mass spectrometry. Overall, the MEDTOX device demonstrated 73 true positives, 4 false positives, and 9 false negative results. The Quidel Triage TOX drug screen device found 68 true positives, 0 false positives, and 14 false negatives. The Quidel Triage® Rapid OXY-BUP-MDMA panel showed 5 true positives, 24 false positives, and 0 false negatives. **Conclusions:** The Profile V MEDTOX device demonstrated the best overall sensitivity and specificity compared to the Quidel Triage TOX drug screen and Quidel Triage® Rapid OXY-BUP-MDMA panel. False positive and negative results are possible with urine drug screens, ultimately the best device may depend on patient population and drugs of interest.

	Confirm Positive by MS	Profile®-V MEDTOX Scan with autoreader		Quidel Triage TOX drug screen with autoreader		Quidel Triage® Rapid OXY-BUP-MDMA visual interpretation	
Drug Class	N	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
AMP n=30	11	73	100	45	100	-	-
MAMP n=25	5	80	100	60	100	-	-
BAR n=26	6	83	100	100	100	-	-
BZO n=32	11	100	100	82	100	-	-
COC n=23	3	67	100	67	100	-	-
THC n=23	3	100	100	67	100	-	-
MTD n=26	6	100	100	100	100	-	-
OPI n=51	28	96	100	93	100	-	-
OXY n=52	5	100	91	NA	NA	100	49
BUP n=26	6	67	100	NA	NA	100	100

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Validation of the Lateral-Flow Dried Blood Spot (DBS) Specimen Delivery Cards for Routine Clinical Laboratory Application

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IntroductionMeasuring circulating protein biomarkers for diagnostic applications is of great significance to many clinical laboratories. Relative to conventional phlebotomy, the Dried Blood Spot (DBS) specimen delivery method offers the potential for very low-cost specimen collection, but lacks suitable validation for routine clinical use, except in newborn genetic testing. This may emanate, in part, from the small amount of specimen collected by this approach relative to the analytical limits of conventional testing methods. With the development of ultrasensitive protein mea-

suring techniques, such as mass spectrometry and Luminex immunobead assays, the DBS platform has newfound potential. Benchmarks for analyte integrity and performance characteristics for this platform are limited or poorly-defined. In this study, we focused on documenting the stability of a series of protein analytes collected through DBS under different storage conditions.

Methods Whole blood was collected in K2EDTA vacutainers from 8 advanced-stage lung cancer patients and immediately spotted on DBS cards utilizing a lateral flow design to resolve cellular component from plasma. Storage variables tested included: time (evaluated at 0, 1, 3, 7, 14, 28 days), impact of a broad-spectrum protease inhibitory (PI) cocktail, and storage temperatures (assessed at 25 °C, 4 °C, and -80 °C). Dried plasma was extracted using optimized condition, consisting of constant agitation of the plasma-dried paper with RIPA buffer for 2h at 4°C. Commercial Luminex kits were then used to asses circulating cytokines (OPN and CEA), growth factors (leptin), Immunoglobulins (autoantibodies for: p53, CCNB1, p16, IMP2, IMP3, SOX2, BIRC5, HIF1α, HSP60, CTAG1B, GAL2, and GAL3), and soluble receptors (sEGFR and suPAR). Significance between conditions were assessed using a Multivariate analysis of variance (MANOVA), whereas statistical agreement was evaluated between the tested conditions using the Intraclass Correlation Coefficient (ICC) test. Coefficient values for agreement were categorized as excellent (>0.9), good (0.75-0.9), moderate (0.5-.0.75), or poor (≤0.5).

Results Storage temperature, PI treatment, and time were tested for significance on a series of protein analytes stored on DBS cards. Storage time did not significantly impact the stability of the seventeen analytes tested, with exception to leptin (p-value=0.006). All markers showed excellent agreement between time points, except CCNB1 and HSP60, which showed only moderate agreement; however, these results were not significant by MANOVA. Both PI treatment and temperature did not have a significant impact on analyte stability by MANOVA. Different storage temperatures had either excellent or good agreement with all analytes tested, with exception of GAL1 and CCNB1, though like before, storage temperature differences were not significant via MANOVA. Finally, the PI cocktail had no influence on analyte stability, with possible exception to CCNB1. While these findings were not statistically significant, this finding suggests possible interference from presence of PI with CCNB1.

Conclusion The DBS platform provides a clinically-suitable platform for plasma delivery with little impact on protein analyte stability for up to 28 days at room temperature. Further investigation into instances where marginal analyte-specific degradation was observed are still ongoing as well as reevaluation with a finger-stick collection protocol.

A-338

A Powerful Clinical Aid in the Diagnosis of Vaginitis to Prevent PTB and Stop STI Transmission

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Background: Globally, it is estimated that 130 million babies are born premature every year, resulting in the deaths of 1.1 million infants. Vaginal microbiome abnormalities that results from Vaginitis are known to be responsible for 50% of all pre-term births (PTBs), which is a major cause of infant mortality, emotional distress and lifelong disabilities. Current screening for symptomatic vaginitis includes wet-mount microscopy, cell culturing or molecular analysis, some of which can be highly inaccurate. Some of these processes also can take days at a time, underlining the need for a rapid diagnostic tool that can accurately assess the health of the patient's vaginal microbiome. The aim of this study was to compare wet-mount microscopic methods with that of Caza Health's vaginal health assessment system, a fluorescence imaging platform coupled with AI analysis software capable of rapidly scanning vaginal specimens to aid physicians in diagnosing symptomatic and asymptomatic vaginitis in women.

Methods: 92 random patients were recruited for the study in collaboration with Edward Via College of Osteopathic Medicine. Vaginal swab samples were analyzed via wet mount microscopy and diagnosed in the clinic. Residual samples were then fixed, dried onto a slide and sent to Caza Health, where they were processed for analysis. Vaginal swab samples were analyzed using the nCyte™ AI system, which produces a high resolution, multi-channel picture for clinical confirmation.

Results: In the 92 patients that were analyzed, 83% were in accordance with the clinic's diagnosis. Of the 17% of samples that were in discordance, 44% contained detectable target organisms that were missed by the wet mount microscopy analysis. The other 66% were cases diagnosed by clinical symptoms alone, meaning nothing was found via wet-mount analysis.

Conclusion: The nCyte™ AI system is a rapid, easy to use system which is intended for use in a point of care setting. The system combines immunofluorescence technology with a portable bench-top automated microscopy system and pathogen search and recognition software to yield a quick and cost-effective method that can be used in a central lab or point of care. The system provides greater accuracy than wet mount microscopy with minimal hands on time. The system will enable physicians to provide patients with a more accurate diagnosis during the office visit and eliminate potential repeat visits.

A-339

Multiplexed Detection of Sweat Cytokine Markers for Real-Time Inflammation Monitoring

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Background: Sweat based wearable sensors have shown a lot of promise as it contains plethora of biomarkers and can be easily integrated with wearable device for real-time continuous monitoring of biomarkers. Among the various biomarkers, sweat cytokines have been studied previously and found to be in comparable range to the blood cytokines. Additionally, real-time sweat cytokine detection helps in monitoring the progression and recovery of inflammation, which is not feasible using the standard blood-based methods. In this work, we demonstrate multiplexed, real-time continuous monitoring interleukin-8 (IL-8) and interleukin-10 (IL-10) in passively perspired eccrine sweat. The developed wearable sensor can detect pro and anti-inflammatory biomarkers that can aid physicians in administering better treatment management methods for various inflammatory conditions. **Methods:** The sweat based sensor was developed with ZnO as the electrode on porous polymeric substrate. The capture probe antibodies were immobilized on the sensor surface via a thiol-cross linking chemistry. Electrochemical impedance spectroscopy technique was used to detect the interaction between the specific antibody and target analyte. The impedance response based on the binding interaction was used to quantify the sensor metrics. **Results:** In this work, a sweat sensor platform for the duplex detection of IL-8 and IL-10 has been demonstrated. Calibration dose response curve was developed with decreasing impedance response for increasing concentrations of target analyte. A limit of detection of 2 pg/mL was obtained with a dynamic range from 2 pg/mL- 200 pg/mL for IL-8. IL-10 demonstrated a limit of detection of 1.5pg/mL with a dynamic range from 1.5- 150pg/mL. High selectivity and specificity of the sensor was achieved with no cross-reactivity to non-specific molecules such as IL-6, IL-1β, TNF-α that are similar. Furthermore, the developed sensor device is of a wearable form-factor that can be worn as a watch to report sweat concentrations continuously. The sensor demonstrated continuous detection for over 30 h in real-time. **Conclusion:** The demonstrated sensing device in this work, will be of tremendous use to monitor the progression and treatment of inflammation and can aid doctors in improvising treatment methods as per patient needs.

A-341

Performance of a Next Generation Urinalysis Dipstick Control

B. Fernández, G. Manuel, P. Onofre, S. Musngi. *Quantimetrix, Redondo Beach, CA*

Background: It is critical that quality control materials are used to verify the performance of clinical assays. A robust QC program ultimately helps to ensure accurate patient diagnoses and appropriate treatment plans. The term urinalysis describes the various diagnostic tests that may be performed on a urine sample. Chemical examination commonly employs the use of urinalysis dipsticks that contain various combinations of reagent pads to semi-quantitatively detect the presence of ascorbic acid, bilirubin, blood, creatinine, glucose, ketones, leukocytes, microalbumin, nitrite, pH, protein, specific gravity, and urobilinogen. These tests are found at most hospitals, clinical laboratories, doctors offices, health clinics, and nursing facilities. Dipsticks may be read visually by comparing the colors that develop on each reagent pad to a chart provided by the strip manufacturer, or by an automated urine dipstick analyzer which helps to provide consistency in the timing and color interpretation regardless of lighting conditions or personnel. Urinalysis dipsticks are a frequently used point-of-care test (POCT). Since refrigeration is not always available near the site of patient care, many POCT devices are designed to be stored and operated at room temperature (RT). Quality control materials that are used to verify the performance of the POCT devices would ideally also have extended RT stability.

Objective: To demonstrate the performance of a next generation urinalysis dipstick control made from real human urine with room temperature stability of at least 2 months in both dipper and dropper-style QC formats.

Methods: Two levels of a human urine control were formulated using proprietary stabilizers and excipients. Level 1 was formulated to test as negative/normal while Level 2 formulated to test as abnormal/elevated for bilirubin, blood, creatinine, glucose, hCG, ketones, leukocytes, microalbumin, nitrite, pH, protein, specific gravity, and urobilinogen. Samples were filled into both 5mL LDPE dropper bottles and 15mL glass dipper tubes. RT stability was evaluated by maintaining samples at 25ºC and testing at several intervals through 240 days. Real-time stability will be followed for up to 2 years at 2-8ºC. SG Roche Chemstrip® 10MD, Siemens Multistix® 10SG, McKesson 10SG, and Henry Schein Urispec® 11-way dipsticks, Siemens Clinitek® Microalbumin 2, and Quidel QuickVue® assay methods were used.

Results: All tests performed maintained the appropriate clinically significant responses for every analyte/assay method/ QC format for up to 240 days at 25ºC, with one exception; ketones were found to remain stable through 65 days at 25ºC. On-going real-time stability at 2-8ºC confirms that all analytes are currently stable through 365 days.

Conclusions: The Next Generation Quantimetrix Urinalysis Dipstick Control formulation shows excellent RT stability of at least two months for all analytes, across multiple assay types, and QC formats. This stability performance exceeds the RT stability of most other human urine-based controls on the market that are formulated with native ketone compounds. Advances to the control formulation have resulted in a marked improvement in the Level 2 urobilinogen reaction which had previously produced atypical color responses on some dipstick methods (McKesson, Acon, Clarity, & Mission brands) that would occasionally be interpreted by the instrumentation as negative.

Tuesday, December 15, 2020

Poster Session: 12:30 PM - 1:30 PM

Pediatric/Maternal

A-344

Newborn Drug Screening: Two Case Reports and Review of Literature

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Background: Identification of newborns exposed to illicit drugs during pregnancy is important to recognize and to legally assess families with substance abuse disorders requiring a close follow-up of the infant by both medical and social services. Drug screening and confirmation in newborns has been traditionally performed on meconium. In recent years, umbilical cord tissue has gained popularity as an alternative specimen for meconium. Here, we present two cases of paired meconium and umbilical cord drug testing leading to different outcomes. The objective of this report is to highlight the potential inconsistencies that may occur with paired testing, and how these results may be interpreted. **Methods:**We report two cases of newborn drug screening of premature infants born to mothers with a history of drug abuse. The drug testing was performed on paired meconium, umbilical cord tissue and/or urine samples. Umbilical cord tissue testing was referred to NMS laboratories (Horsham, PA) which performed analysis using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) followed by confirmation with the same method. Meconium samples were analyzed by LabCorp laboratories (Louisville, KY) using immunoassay followed by confirmation by HPLC-MS/MS testing. Urine drug screening was performed on both maternal and newborn samples at University of Louisville Hospital (ULH) core laboratory using an immunoassay-based method. Confirmation of urine drug screening was confirmed with HPLC-MS/MS send-out to LabCorp. Each specimen was tested for amphetamine, methamphetamine, opiates, cocaine, cannabinoids and phencyclidine (PCP). **Results:**Case I: An early term infant was born at 38 weeks and 3 days gestation to a 35-year-old mother who had a history of drug abuse of amphetamines and opiates. No other maternal information was available. Initially, the urine drug screens performed at ULH on both the mother and the baby were positive for amphetamine and/or methamphetamine. The maternal results were further confirmed with LC-MS/MS. Meconium drug screening indicated the presence of amphetamine, methamphetamine, morphine, codeine, benzoylecgonine and m-OH-benzoylecgonine. In the umbilical cord, codeine was not detected, and fentanyl and norfentanyl gave positive results. Case II: A preterm infant was born at approximately 36 weeks gestation to a 33-year-old mother who had a history of drug abuse, stillbirth, and was diagnosed with hepatitis C. The mother had no prenatal care or ultrasounds for this pregnancy. Maternal urine drug screening was positive for amphetamine, methamphetamine and buprenorphine; the newborn's urine sample was not available at that time. No confirmation was performed for urine UDS. LC-MS/MS toxicology testing on meconium revealed the presence of amphetamine, methamphetamine, buprenorphine and its metabolite norbuprenorphine. Umbilical cord did not detect any buprenorphine or norbuprenorphine. **Conclusion:**The two cases have revealed different sensitivities for drug detection in meconium and umbilical cord tissue. Meconium, to a significant extent, provides higher sensitivity, and is likely to remain the specimen of choice when sensitivity is of greatest importance. These results can help clinicians, laboratorians, and epidemiologists to (1) select the most appropriate test to confirm a suspected drug exposure and (2) interpret discordant results when testing is performed in multiple matrices.

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Clinical Value of Serum Amyloid A (SAA) Protein, High Density Lipoprotein-Cholesterol (HDL-C) and Apolipoprotein-A1 (Apo-A1) in the Diagnosis and Follow-Up of Neonatal Sepsis

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Background: Sepsis is a major cause of morbidity and mortality in neonates and may also have severe long-term consequences, including adverse neurodevelopmental outcomes and possibly increased risk for the later development of asthma and allergy. SAA protein, a phylogenetically highly conserved protein synthesized predominantly by the liver in response to proinflammatory cytokines, has been proven as an acute phase reactant with its blood concentrations increasing up to 1000-fold, or more, following appropriate stimuli. In the circulation, SAA associates with HDL-C during the acute phase response and becomes its main apolipoprotein by displacing Apo-A1. In septic adults, SAA has been recognized as a specific and accurate biomarker, whereas prominent decreases in serum HDL-C and Apo-A1 levels at the acute phase of sepsis have also been reported. We aimed to study the performance of SAA, HDL-C and Apo-A1 levels in the identification and monitoring of neonatal sepsis. **Methods:** This prospective study included 113 full-term septic neonates and 68 healthy neonates (controls). Blood samples were drawn serially in septic neonates at enrollment and on days 1, 3 and 7, and once in controls, for SAA, HDL-C and Apo-A1 determination along with other hematological and blood chemistry parameters. **Results:** At enrollment, SAA levels were significantly higher in septic neonates in comparison with controls (median 50.7 vs. 3.5mg/L, $p<0.001$); HDL-C and Apo-A1 levels were significantly lower in patients than in controls ($p<0.001$ and $p=0.006$, respectively). SAA levels were higher in culture-positive compared to culture negative sepsis (median 202.0 vs. 14.2mg/L, $p<0.001$). HDL-C and Apo-A1 levels did not differ significantly between culture-positive and culture-negative sepsis. In patients who improved clinically within 48 hours following enrollment, the initially increased SAA levels declined rapidly, by more than 90%, in a time-period of 3 days, whereas the initially decreased HDL-C and Apo-A1 levels remained persistently low or even lower during the same time period.ROC analysis of SAA levels at enrollment resulted in significant areas under the curve (AUC) for detecting sepsis [AUC=0.929 (95%CI 0.885-0.973), $p<0.001$] and also for discriminating between culture-positive and culture-negative sepsis [AUC=0.933 (95%CI 0.882-0.984), $p<0.001$]. The combination of HDL-C and Apo-A1 with SAA increased its diagnostic performance. Furthermore, serial SAA levels following enrollment could indicate clinical response in septic neonates. **Conclusions**: According to the results of this study, SAA concentrations are increased, whereas HDL-C and Apo-A1 levels are decreased, at the acute phase of neonatal sepsis in comparison with healthy controls. The diagnostic value of baseline SAA levels for neonatal sepsis was very good, similar to that of CRP; moreover, baseline SAA levels could discriminate between culture-positive and culture-negative sepsis. On the contrary, HDL-C or Apo-A1 levels neither were accurate diagnostic markers of neonatal sepsis, nor had any value in discriminating culture-positive than culture-negative sepsis. Furthermore, in septic neonates, follow-up SAA levels could better indicate clinical response in comparison with HDL-C or Apo-A1 levels. SAA seems to be a valuable biomarker for identification and monitoring of neonatal sepsis, and also for discriminating between culture-positive and culture-negative sepsis. HDL-C and Apo-A1 could be used as complementary markers.

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Elevated Circulating Endothelial Microparticles in Prepubertal Children Born Prematurely: Further Evidence of the Cross-Talk between Prematurity and Atherosclerosis

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Background: Several studies have shown that children and/or adults who were born prematurely display cardiovascular and metabolic alterations. Although endothelial

dysfunction is the initial step towards cardiovascular disease, the influence of prematurity on the development of endothelial injury has not been clarified yet. Circulating endothelial microparticles (EMPs) are described as small membrane vesicles (0.1-1.0μm), released from endothelial cells in response to stimuli such as inflammatory activation, apoptosis or injury). EMPs act as novel biomarkers of endothelial activation and damage. In this context we aimed to investigate circulating endothelial microparticles (EMPs) in prepubertal children born prematurely. **Methods:** A case-control study was conducted and 104 prepubertal children were enrolled; 52 preterm and appropriate for gestational age (AGA), and 52 born at term and AGA, matched for age and gender with preterm, as the control group. The main outcome was the measurement of circulating CD62E(+), CD144(+) and CD31(+)/CD42b(-) EMPs, by means of flow cytometric techniques, in preterm born children compared to controls. Body mass index/BMI, waist-to-hip ratio/WHR, neck circumference, systolic/SBP and diastolic/DBP blood pressure, fasting glucose and insulin, lipid profile, common carotid/cIMT and abdominal aortic/aIMT intima-media thickness, endothelium-dependent brachial artery flow-mediated dilation/FMD, as well as echocardiographic parameters, were also assessed. **Results:** In preterm born children, circulating CD62E(+), CD144(+) and CD31(+)/CD42b(-) EMPs were significantly higher compared to controls ($p<0.01$). Significant correlations among EMPs subpopulations were observed; levels of CD62E(+) EMPs correlated positively with CD144(+) ($rs=0.80$, $p<0.001$) and CD31(+)/CD42b(-) EMPs ($rs=0.56$, $p<0.001$); CD144(+) were correlated with CD31(+)/CD42b(-) EMPs ($rs=0.48$, $p<0.001$). In the total study population, circulating CD62E(+) EMPs were correlated significantly with gestational age, birth weight, WHR, and mean pressure and velocity of pulmonary artery; circulating CD144(+) EMPs correlated with gestational age, birth weight, SBP, and mean pressure and velocity of pulmonary artery; circulating CD31(+)/CD42b(-) EMPs correlated with WHR, DBP, glucose levels, mean cIMT, and mean pressure and velocity of pulmonary artery. In multiple regression analysis, preterm birth was recognized as an independent predictor for each EMP subpopulation studied; more specifically, preterm birth correlated significantly with CD62E(+) ($\beta=0.25$, $p=0.02$), CD144(+) ($\beta=0.40$, $p<0.001$) and CD31(+)/CD42b(-) EMPs ($\beta=0.30$, $p=0.004$). Moreover, the mean pressure of pulmonary artery was independently correlated with CD62E(+) EMPs ($\beta=0.21$, $p=0.04$); the mean velocity of pulmonary artery was independently correlated with CD144(+) EMPs ($\beta=0.23$, $p=0.02$); glucose levels and the mean pressure of pulmonary artery were independently correlated with CD31(+)/CD42b(-) EMPs ($\beta=-0.24$, $p=0.02$ and $\beta=0.24$, $p=0.03$, respectively). **Conclusions:** Circulating numbers of EMPs are increased in prepubertal children born prematurely in comparison with their peers born at term. Prematurity is associated with indices of endothelial dysfunction and increased cardiovascular risk in children of prepubertal age. As data accumulate, we propose that an early, constant, and prolonged follow-up program for prematurely born individuals should be implemented for the prevention and early detection of disorders leading to cardiovascular disease in later life. Whether assessment of circulating EMPs is warranted in individuals born prematurely, in clinical practice, as a new tool for non-invasive evaluation of endothelium and risk stratification, or as a target for novel therapeutic options, remains to be defined in future investigations.

A-347

A Study of the Effect of Cotton Balls used in Urine Collection on the Chemical Urinalysis

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Background: Cotton balls are frequently utilized for urine collection in neonates and infants for chemical urinalysis, however, most automated UA device manufacturers have not validated this urine collection approach and accompanying product inserts specifically state this is not an acceptable specimen source. The objective of this study was to determine if adsorption of urine analytes to cotton and/or contamination affects chemical UA results on the Iris iChemVelocity automated UA system.

Methods: 40 urine specimens collected from adults with conventional urine cups or tubes were selected and stored in the refrigerator after routine UA. For each urine specimen, a 3-ml aliquot was made in a UA Kova tube for direct testing (tube 1) and two additional 4-ml aliquots (tubes 2 and 3) were made. A cotton ball was placed at the bottom of tubes 2 and 3 (approx. 0.10 g cotton/mL urine). After 20 minutes (tube 2) and 2 hours (tube 3) incubation at refrigerator temp (approx. 4ºC), the cotton balls were inserted into a 10-ml syringe and urine was expelled into a new Kova tube by inserting the plunger. All three sets of specimens were brought to room temperature for 30 min before testing on the Iris iChemVelocity automated analyzer. Results were analyzed by EP Evaluator software using McNemar-Bowker test for symmetry of a 4 x 4 or 5 x 5 contingency table, depending on the levels of reportable results, to determine whether cotton consistently caused biased results.

Results: Out of a total of forty patient urine specimens, 16 were positive for glucose, 27 positive for protein, 4 positive for bilirubin, 7 positive for urobilinogen, 30 positive for blood, 14 positive for ketone, 6 positive for nitrite and 22 positive for leukocyte esterase. After both 20-min and 2-hr cotton ball absorption, concordance of results of cotton absorbed and untreated urine were ≥ 95% for pH, glucose, bilirubin, urobilinogen, ketone and nitrite. McNemar-Bowker test showed that both 20-min and 2-hr of cotton absorption consistently caused lower level positive results for blood and leukocyte esterase. Of 30 positive blood samples, 19 were decreased by one level after cotton absorption, and one was decreased by two levels from LARGE to SMALL after 2hr of absorption. Of 22 positive leukocyte esterase samples, 12 were decreased by one level after cotton ball absorption, and one was decreased by two levels from SMALL to NEGATIVE after 2hr incubation. Although 5 and 8 of 27 positive protein samples were decreased by one level after 20-min and 2-hr of cotton absorption respectively, McNemar-Bowker test indicated only 2-hr of absorption caused a consistent change. By Deming Regression, specific gravity, urine sodium, potassium and chloride were well correlated without bias between untreated urine and urine after cotton absorption.

Conclusion: UA results for urine collected with cotton balls show minimal bias compared with untreated urine for pH, glucose, bilirubin, urobilinogen, ketone, nitrite, electrolytes and specific gravity in chemical UA. However, negative bias of 1 or 2 levels or falsely NEGATIVE results for blood, leukocyte esterase and protein may occur.

A-349

CALIPER Pediatric Reference Value Distributions for Plasma Cytokines and Chemokines and Establishment of Age-Stratified Reference Intervals on the ProteinSimple Ella Multiplex Immunoassay Platform

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Background: Cytokine and chemokine measurements help elucidate disease pathobiology, assist in diagnostic and treatment decisions, and aid in assessment of therapeutic response. Reference values provide a baseline for which laboratory test results are compared to in clinical practice. Appropriate interpretation of cytokine and chemokine test results requires appropriate reference values and decision limits based on a healthy population. Since growth and development can markedly influence immune response, accurate platform-specific reference intervals established from a healthy pediatric population are essential for cytokine and chemokine result interpretation. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) program has previously reported a comprehensive database of pediatric reference intervals for over 180 biochemical and immunochemical markers. The objective of this study was to establish pediatric reference values and cut-offs for 8 plasma cytokines and chemokines in the CALIPER cohort for the first time to aid in the interpretation of cytokine results. **Methods:** Healthy children and adolescents (n=311), aged 1-19 were recruited as part of the CALIPER study with informed consent. Analytes were measured in EDTA plasma using multiplex immunoassay panels on the ProteinSimple® Ella™ System (Bio-Techne, Minneapolis, MN, USA) (Panel 1: CD163, CXCL-9/MIG, IFN- γ, TNF-α; Panel 2: IL-1β, IL-6, IL-10, IL-18).. Pediatric reference intervals, representing the 2.5th and 97.5th percentiles, were calculated according to CSLI C28-A3 guidelines. Partitions based on age and/or sex were determined visually and statistically evaluated using the Harris and Boyd method. After removal of outliers using the Tukey or adjusted Tukey method, reference intervals were calculated using the non-parametric rank method if the sample size was larger than 120 and the robust method if the sample size was less than 120. Additionally, 75th and 95th percentile cut-offs were determined for each marker. **Results**: Complex patterns of change were observed in numerous analyte concentrations across the age continuum. The patterns were classified into 1 of 3 categories: (a) consistent concentrations throughout age and sex: IL-6, and IFN-γ (b) gradual concentration decrease with age: CD163, TNF-α, CXCL-9/MIG, and IL-10 (c) sharp rise in concentrations during ages 4-14 with significantly lower concentrations in earlier and later age groups for IL-1β and IL-18. Many analytes showed dynamic changes in concentration requiring age partitioning. Unique intervals were required within the first 8 years of life for CXCL-9/MIG, IL-10, and TNF-α. CD163, IL-18 and IL-1β required 3 age partitions, while IL-6, and IFN-γ required only one. CD163 demonstrated sex-differences in ages 8-13. IL-6, IL-1β, and IFN-γ were undetectable in 12%, 18%, and 49% of subjects, respectively. The remaining markers were detectable in all samples tested. **Conclusions**: This study provides robust reference values and percentile cut-offs for 8 cytokines and chemokines in a healthy pediatric population on the ProteinSimple Ella platform. Complex

profiles were observed for cytokines and chemokines requiring establishment of age and sex-specific reference intervals. These reference intervals will allow for improved laboratory assessment of pediatric patients using this assay platform worldwide. The reference values and cut-offs proposed herein should be verified for each analytical platform and local population as recommended by CLSI.

A-350

The Effect of Acidification of Urine on Electrolytes in Infants Receiving High Calcium/Phosphorous Nutritional Formula

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Background: The concentration of calcium, magnesium, and phosphorous are routinely measured in urine for the evaluation of renal excretion in the context of a wide variety of disease states. It has been a longstanding practice to acidify urine samples to a pH<2 prior to analysis to dissolve potential salt precipitates that can lead to erroneously low measurements. In the past decade, however, the necessity of this practice has been called into question as the bias between acidified and non-acidified urine is not clinically significant. These studies, however, have not evaluated the significance of urinary acidification in infants born prematurely, despite this demographic's high risk for renal pathology including nephrocalcinosis and secondary hyperoxaluria exacerbated by the feeding of high calcium/phosphorous formula. The objective of this study was to evaluate the analytical and clinical significance of electrolyte bias in acidified versus non-acidified urine, with a particular focus on infants receiving nutritional formula. **Methods:** Residual non-acidified urine specimens (n=97) were split into two aliquots. One aliquot was acidified by the addition of nitric acid to pH<2 and incubated at room temperature for 1 hour. The remaining aliquot was not acidified. Both aliquots were then centrifuged to remove sediment and analyzed for calcium, magnesium, and phosphorous using a Roche Diagnostics Cobas c702 analyzer. The difference between electrolyte concentrations from acidified and non-acidified was calculated. To determine the clinical significance of biases, the reference change value (RCV) for each electrolyte was calculated for the 95% confidence interval using the equation $RCV = 2.77 * (((CV_A\text{^}2)+(CV_I\text{^}2))\text{^}0.5)$. The analytical variation, CV_A, was determined by replicate quality control material analysis while the intra-individual biological variation, CV_I, was obtained from published literature. The final RCVs were 101.44%, 104.83% and 73.76% for calcium, magnesium, and phosphorous, respectively. The use of infant nutritional formula was determined by retrospective medical chart review. **Results:** The mean % difference (SD) of acidified versus non-acidified samples was 0.85% (6.69%), -1.98% (7.06%), and -1.16% (2.86%) for calcium, magnesium, and phosphorous, respectively. No samples exceeded the RCV. Among infants receiving nutritional support (n=8, 9, 9), the mean % difference (SD) was 5.25% (20.50%), -1.29% (10.15%), and 2.69% (6.33%) for calcium, magnesium, and phosphorous, respectively. Of the infants specifically receiving high calcium/phosphorous nutritional formulas (n = 4), 2 (50%) samples were outliers with calcium biases of 26.92% and 36.71%, however the same effect was not seen with magnesium or phosphorous. **Conclusions:** No clinically significant differences were observed in calcium, magnesium and phosphorous measurements when comparing acidified and non-acidified urine samples. The most significant calcium biases were observed in some infants receiving high calcium/phosphorous nutritional formulas, however the RCV was not exceeded. Providers and laboratories should be aware that this patient population is particularly sensitive to calcium under recovery when non-acidified specimens are used for analysis.

A-352

Evaluation of the Pico-Anti-Müllerian Hormone (AMH) Assay in Patients undergoing In Vitro Fertilization and Examining Its Value as a Predictor of Ovarian Hyperstimulation Syndrome

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Objective: Anti-Müllerian hormone (AMH) is used clinically as a biomarker of ovarian reserve. AMH is also being evaluated as a predictor of response to controlled ovarian stimulation (COS) during treatment with assisted reproductive technologies (ART). Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening complication of COS and there are few reliable biomarkers. AMH is of particular interest as a predictive biomarker of OHSS. Patients with a high ovarian reserve, reflected by high AMH and antral follicle count (AFC), are often at greatest risk for OHSS. Our study aims to evaluate the performance of a new automated pico AMH ELISA for the prediction of OHSS during COS. **Study design**: We performed retrospective chart review of patients undergoing COS who had AMH measured. Response to COS was defined as poor (<4 oocytes retrieved), normal (4-15 oocytes), or hyper-response (>15 oocytes). AMH was measured by ELISA (pico AMH, Ansh Laboratories). Statistical analysis was performed by one-way ANOVA, and results considered significant when $p < 0.05$. Clinical performance of AMH and AFC for prediction of OHSS was assessed by receiver operating characteristics (ROC) analyses. **Results:** A total of 78 COS cycles were included with a mean patient age of 35 (±4.6) years and BMI of 26.1(±8.9) kg/m^2. Within the study group, 3 patients were poor responders (3.8%), 29 were normal responders (37%) and 37 (47%) were hyper-responders. There was no significant difference in age by COS response. Both AMH and AFC values differed significantly ($p<0.0002$, $p<0.00001$) between normal 2.66(±2.47) ng/ml and hyper-responders 5.82(±4.24) ng/ml. Following COS, 8 patients (10%) were diagnosed with OHSS, all of whom were hyper-responders. There were significant differences in AMH ($p<0.00001$) and AFC ($p<0.0009$) between OHSS and non-OHSS patients. However, in hyper-responders, only AMH differed significantly ($p<0.0004$) between OHSS and non-OHSS. Clinical performance of AMH for prediction of OHSS was assessed by ROC analysis and compared to clinical performance of AFC. An AMH cutoff value of 3.6 ng/ml was chosen to optimize sensitivity (87.5%), yielding specificity of 73.5% (in all patients) and 60% (in hyper-responders). AFC was significantly less specific with a specificity of 25%(in all patients) and 49.3%(in hyper-responders) at the same sensitivity level. **Conclusions:** We report for the first time the clinical performance of the picoAMH assay in prediction of OHSS in patients undergoing COS using a cutoff of 3.6 ng/mL. Our study suggests that picoAMH is a better predictor of OHSS than AFC.

A-353

Diagnostic Concordance between BNP and NT-proBNP in Pediatric Patients

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Background: B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP) are essential biomarkers for evaluation of heart disease in pediatric cardiac patients. Circulating concentrations of each peptide vary based on factors such as age, gender, and assay type. Pediatric NT-proBNP reference intervals are not well defined and concordance between BNP and NT-proBNP in the evaluation of pediatric patients has not been described. The objective of this study was to assess the concordance between BNP and NT-proBNP using reference intervals adopted for the patient population at an academic tertiary care pediatric hospital. **Methods:** Routine BNP testing was ordered for 274 individual patient samples (aged 18 and under; 148 male, 126 female) over a 21-week period. BNP (Abbott Architect) and NT-proBNP (Roche Cobas) testing was performed simultaneously on each sample. Pediatric reference intervals and upper limits for BNP and NT-proBNP were used to evaluate diagnostic concordance based on age group. Statistical analyses were performed using GraphPad Prism. Pediatric NT-proBNP reference limits were derived from literature reports and incorporated at St. Louis Children's Hospital. **Results:** Deming regression of BNP and NT-proBNP revealed a slope of 13.63 (95% CI, 10.35 - 16.92) and y-intercept of -977.8 (95% CI, -2063 - 107.2) for all age groups (r^2, 0.7650; S_{yx}, 5118). By age group, concordance between BNP and NT-proBNP by weighted Cohen's kappa was 1.0 for age 0 to 10 days (n = 5), 0.23 for age 11 to 30 days (95% CI, 0 - 0.62; n = 12), 0.82 for age 31 days to 1 year (95% CI, 0.67 - 0.97; n = 84), 0.81 for age 1 to 2 years (95% CI, 0.57 - 1.0; n = 22) and 0.73 (95% CI, 0.64 - 0.86; n = 151) for age 2 to 18 years (n = 151). Overall, a concordance of 0.80 (95% CI, 0.73 - 0.87; n = 274) was calculated between BNP and NT-proBNP in pediatric patients (n = 274). In patients with multiple, serial tests ordered (n = 22) 31% of measurements exhibited a discordant increase or decrease in BNP versus NT-proBNP compared to previously reported values (e.g., patient has an increase in BNP and decrease in NT-proBNP). **Conclusions:** There is poor correlation between BNP and NT-proBNP but moderate clinical concordance between BNP and NT-proBNP in the pediatric population studied. Concordance among pediatric patients was higher than in previously published adult studies.

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CLSI-Based Verification of CALIPER Pediatric Reference Intervals for Endocrine and Fertility Hormones on the Abbott Alinity Platform

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Background: Reference intervals (RIs) are essential for accurate test result interpretation. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALI-

PER) has developed age- and sex-specific RIs for over 170 biomarkers on several analytical platforms. In the current study, published CALIPER RIs for nine immunoassays on the Abbott ARCHITECT platform were assessed for verification on the new Abbott Alinity platform using serum samples from healthy children and adolescents.

Methods: Based on Clinical and Laboratory Standards Institute (CLSI) guidelines, 100 serum samples from the CALIPER cohort were analyzed for nine immunoassays (i.e. free thyroxine, free triiodothyronine, total thyroxine, total triiodothyronine, follicle stimulating hormone, luteinizing hormone, progesterone, and 25-hydroxy Vitamin D) on the Abbott Alinity platform. The percentage of test results falling within published ARCHITECT confidence and reference limits was determined for each analyte. If ≥ 90% of test results fell within the confidence limits of published CALIPER RIs, they were considered verified.

Results: Of the nine immunoassays assessed, eight met the criteria for RI verification with test results from ≥ 90% of healthy serum samples falling within the published ARCHITECT confidence limits. CALIPER RIs previously published for free thyroxine on the ARCHITECT platform did not verify on the Alinity platform, with only 67% of reference samples falling within published confidence limits.

Conclusion: Our data demonstrate excellent concordance between the Abbott ARCHITECT and Alinity immunoassay platforms, as well as the robustness of previously established CALIPER RIs for nine thyroid and sex hormones, eliminating the need for de novo RI studies for most parameters. These results can be expected to facilitate pediatric test result interpretation in institutions using the new Alinity platform.

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CLSI-Based Verification of CALIPER Pediatric Reference Intervals for Routine Biochemical Assays on the Abbott Alinity Platform

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Background: The quality of laboratory service is dependent on the accuracy of reference intervals (RIs). As new analytical platforms and reagents continue to be developed/improved, previously established RIs must be verified as recommended by the Clinical and Laboratory Standards Institute (CLSI). The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has established comprehensive RIs for many biomarkers on several analytical platforms. In this study, published CALIPER RIs for 11 chemistry assays on the Abbott ARCHITECT were assessed for verification on the newer Alinity platform.

Methods: Based on CLSI guidelines, 100 healthy pediatric serum samples from the CALIPER cohort were analyzed for 11 chemistry assays (i.e. aspartate aminotransferase (with and without pyridoxal phosphate, albumin (bromocresol green and purple), alkaline phosphatase, apolipoprotein A1, apolipoprotein B, direct bilirubin, total bilirubin, calcium, and carbon dioxide) on the Abbott Alinity platform. The percentage of test results falling within the published ARCHITECT confidence and reference limits was determined for each analyte. If test results for ≥ 90% of healthy serum samples fell within the confidence limits of published CALIPER RIs, the RIs were considered verified.

Results: Of the 11 chemistry assays assessed, six met the criteria for RI verification (including aspartate aminotransferase (with and without pyridoxal phosphate), alkaline phosphatase, apolipoprotein A1, apolipoprotein B, and carbon dioxide). Reference values obtained for albumin (bromocresol purple and green) and calcium demonstrated marked differences on the Alinity platform, with over 50% of results falling outside the previously published ARCHITECT RIs. Direct and total bilirubin also did not verify, but closely approached the outlined criteria.

Conclusion: In the current study, CALIPER RIs originally established on the ARCHITECT platform were verified on the Alinity platform for several chemistry assays. These data demonstrate good concordance for these assays between the Abbott ARCHITECT and Alinity platforms and will assist in the implementation of the Alinity platform at pediatric healthcare institutions, globally. For other assays, direct RI studies are planned to establish new Alinity RIs.

A-356

CALIPER Reference Standards for Hematology Parameters on the Beckman Coulter DxH 520 Analytical Platform (Physician Office Instrument): Improving Laboratory Test Interpretation in Children

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Background: Diagnosis and monitoring of hematological disorders in children require laboratory testing and appropriate reference standards for accurate test result interpretation. Unfortunately, reliable normative data on modern systems are not currently available. The objective of this study was to establish comprehensive age- and sex-specific reference intervals for hematologic parameters in the CALIPER cohort of healthy children and adolescents.

Methods: A total of 536 healthy children and adolescents (birth to 21 years) were recruited with informed consent and whole blood samples were analyzed for 27 hematologic parameters on Beckman Coulter DxH 520 system. Age- and sex-specific pediatric reference standards were established. Reference values obtained on DxH 520 were also compared with data obtained on a larger laboratory-based instrument (DxH 900).

Results: Most hematological parameters showed significant age-specific and/or sex-specific changes during growth and development. Of the 27 hematological parameters, all except four (mean corpuscular hemoglobin concentration, basophil percentage, low hemoglobin density, immature cell percentage) required age-partitioning and eight required sex-partitioning.

Conclusion: This study establishes a robust pediatric hematology reference database that will assist in more accurate test result interpretation. Our data clearly demonstrates significant variation in hematological parameter concentrations in children and adolescents, necessitating the use of pediatric-specific reference standards.

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CALIPER Reference Standards for Hematology Parameters on the Beckman Coulter DxH 900 Analytical Platform (Core Hematology Analyzer): Improving Laboratory Test Interpretation in Children

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Background: Accurate hematological test interpretation based on normative reference standards is critical to ensure appropriate clinical decision-making. However, healthy pediatric reference data for most hematology parameters is lacking. To address this gap, this study establishes age- and sex-specific hematological reference standards in the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) cohort of healthy children and adolescents.

Methods: Fresh whole blood samples collected from a total of 566 healthy children and adolescents (birth to <21 years) with informed consent were analyzed for 47 hematological parameters on Beckman Coulter DxH 900. Age- and sex-specific reference standards were calculated based on the Clinical and Laboratory Standards Institute guidelines.

Results: Reference value distributions for the majority of hematology parameters demonstrated dynamic changes across the pediatric age range with significant age-specific differences observed for 39 of the 47 parameters examined. Sex-specific differences were also observed for eight hematological parameters, primarily during and after puberty.

Conclusion: This study establishes a robust database of pediatric reference standards for 47 hematological parameters in the CALIPER cohort for the first time. These comprehensive reference value datasets report potentially important and physiologically relevant trends in hematological markers, clearly demonstrating the need for pediatric reference standards for hematological test interpretation.

A-361

Serum Concentrations of Ferritin and Soluble Transferrin Receptor during Normal Pregnancy

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Background: The timely diagnosis and treatment of anemia during pregnancy is critical because it is associated with poor outcomes such as increased risk for pre-term

delivery and low birth weight. Iron deficiency anemia is the most common cause and is traditionally diagnosed using a serum ferritin level <15 ng/mL. However, because it is an acute phase reactant serum concentrations may be elevated and superficially appear to be within the normal range in patients experiencing inflammation. Soluble transferrin receptor (STfR) may be a more reliable biomarker because it is not an acute phase reactant and it reflects the degree of iron requirement in relation to supply. We aimed to measure the serum concentrations of ferritin and STfR throughout normal pregnancy to develop normal ranges to facilitate the diagnosis of prenatal microcytic anemia.

Methods: A total of 120 pregnant women with normal blood hemoglobin levels (WHO/ACOG definitions) were tested for serum levels of ferritin, C-reactive protein (CRP), and STfR. Forty age-matched, non-pregnant women with normal blood hemoglobin levels were also tested as a control group. Serum ferritin and CRP levels were determined using the Beckman Coulter AU 5800 chemistry analyzer and STfR measurements were performed on the Siemens BNII. Descriptive statistics (mean, median, and standard deviation) were determined for each group. Two-tailed t-tests were used to determine statistically significant differences between the non-pregnant control group and pregnant patients in each trimester.

Results: Mean age of study patients was 29 ± 6 years. The mean values ± standard deviation in the control group, first, second and third trimesters, respectively, were as follows: ferritin 59 ± 38, 58 ± 41, 33 ± 22, 28 ± 23 ng/mL; CRP 0.47 ± 0.37, 0.52 ± 0.52, 0.72 ± 0.54, 1.28 ± 1.33 mg/dL; and STfR 1.23 ± 0.35, 1.06 ± 0.37, 1.09 ± 0.30, 1.37 ± 0.38 mg/L. Statistically significant differences were observed for both ferritin and CRP between the non-pregnant control group and women in the second (P=0.0004; 0.001) and third (P < 0.0001; < 0.0001) trimesters of pregnancy. No statistically significant differences were observed for STfR between the control and pregnant groups; however, use of the STfR/ferritin ratio did reveal differences.

Conclusion: Serum concentrations of ferritin, CRP, and STfR were assessed in each trimester of pregnancy. The CRP data supports previous studies demonstrating changes in the inflammatory profile throughout gestation. A statistically significant difference in ferritin levels between the control group and the second and third trimesters suggests that a pregnancy-specific reference interval may be warranted. Differences were observed in the STfR levels between trimesters though they did not prove to be statistically significant. Our findings support previous studies in which lower sTfR values were obtained in the first two trimesters compared with the third trimester or non-pregnant women. This data suggests that a single reference interval for STfR may be acceptable for both pregnant and non-pregnant females. Use of the STfR/ferritin ratio may provide a means of further stratification and warrants further investigation.

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Evaluation of Cannabis Use in Second Trimester Maternal Quadruple Screen Specimens

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Background: According to the National Institute on Drug Abuse (NIDA), cannabis use during pregnancy has more than doubled during the period of 2002 to 2017 (3.4% to 7.0%; NIDA, 2019). Despite limited research, recent studies have shown that tetrahydrocannabinol (THC), the major psychoactive ingredient in cannabis, is linked to higher rates of stillbirth, preterm birth, fetal growth restrictions, and developmental issues. Self-report of substance use during pregnancy may not provide an accurate representation of in utero exposure due to underreporting. The aim of the current study was to evaluate the prevalence of cannabis use in maternal quadruple (Quad) screen specimens received at our laboratory. **Methods:** Residual human serum specimens previously tested for maternal Quad screens (n=271) were obtained from storage (-20°C) and de-identified according to an IRB-approved protocol. Inclusion criteria for these specimens were a negative Quad screen, singleton pregnancy, and the absence of insulin-dependent diabetes, medications associated with fetal defects, family history of neural tube defects, or in vitro fertilization pregnancies. Specimens were selected to specifically include disclosed cigarette (i.e. tobacco) smokers (n=71), disclosed non-smokers (n=100), and specimens with unknown smoking status (n=100) based on cigarette smoking history provided on patient history forms for the Quad screen. 11-Nor-9-carboxy-THC (THC-COOH) was measured in these 271 specimens by an in-house developed quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. A THC-COOH cutoff of ≥5 ng/mL was used to classify specimens as containing THC-COOH. Presence of THC-COOH was also compared to categorized probable tobacco smoking status, as determined by corresponding results from cotinine (COT) measurements in these specimens using an LC-MS/MS method. **Results:** For disclosed cigarette smokers, 29.6% (n=21 of 71) of specimens were positive for THC-COOH, as compared to 13.0% (n=13 of 100) in disclosed non-smokers. For those with undisclosed cigarette smoking status, 5.0% of specimens (n=5 of 100) had THC-COOH present. Similar prevalence of detectable THC-COOH (29.5%; n=23 of 78) was observed in specimens that were categorized as probable tobacco smokers (COT >10 ng/mL). Specimens categorized as being from patients not exposed to tobacco (COT <2 ng/mL) had a lower prevalence of detectable THC-COOH (7.6%; n=14 of 184). **Conclusion:** THC-COOH was present in a higher percentage of specimens from both disclosed and probable cigarette smokers than non-smokers in our study population. The potential impact of cannabis use on maternal serum screening markers is unknown. Additional studies may be warranted given the prevalence of cannabis use observed in population-based reports.

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An Indirect Reference Interval Study to Establish Pediatric Reference Intervals for Alkaline Phosphatase

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Background: Children tend to have higher serum alkaline phosphatase (ALP) than adults due to active bone growth. The default use of adult ALP reference intervals (RIs) for patients of all ages may mask the recognition of low ALP in children, which is a hallmark finding in hypophosphatasia. In this study, we used retrospective laboratory data to indirectly establish age- and sex-specific ALP RIs, and we compared our results to RIs provided by the assay manufacturer and the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER). **Methods**: We retrospectively reviewed ALP results reported from the laboratory for patients between 0-18 years old between January-April 2019. ALP was performed on a Roche cobas c702 analyzer, with adult RIs defined as 35-104 U/L for females and 40-129 U/L for males. We included only the last chronological result for cases of duplicate patients. Outliers were removed based on a 1.5 x interquartile range method. RIs were determined by a nonparametric 95th percentile method.**Results and Discussion**: The derived RIs are presented in Table 1. The derived RIs were wider in distribution than the CALIPER RIs, likely because we could not reliably exclude all unhealthy patients from our data. However, the derived RIs were notably closer to the CALIPER age-specific RIs than the manufacturer's adult RIs. The percentage of pediatric patients who would have been reclassified as within versus outside of the RI upon adoption of our derived RIs rather than the default adult RIs ranged from 4% to 93% among the age brackets, with the largest impact seen in the younger age brackets. **Conclusio**n: Based on the results of our study, we recommended that our hospital implement pediatric age- and sex-specific reference intervals for ALP rather than use adult reference intervals for all.

TABLE 1.

	Females		Males	
Age	**Alkaline phosphatase (U/L)**	**N**	**Alkaline phosphatase (U/L)**	**N**
0 - <15 d	58 - 367	49	71 - 404	53
15 d - <1 y	74 - 496	53	108 - 566	48
1 - <10 y	100 - 416	175	117 - 378	182
10 - <13 y	86 - 347	79	137 - 430	65
13 - <15 y	48 - 192	61	44 - 355	60
15 - <17 y	54 - 163	108	61 - 267	97
17 - <19 y	51 - 130	147	54 - 145	140

A-365

Capillary Lead Screening: Retrospective Review of False Positive Rates with the LeadCare Ultra

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Background:It is a common practice to screen blood lead levels (BLLs) using capillary samples, with confirmatory testing performed on a venous sample by a reference method if elevated. We recently implemented this practice by validating the LeadCare Ultra® device for in-house capillary lead screening, with venous confirmation sent to a reference laboratory for testing by ICP-MS. Although the LeadCare Ultra® is

not a point-of-care (POC) device, we perform repeat analysis of all elevated capillary BLLs to confirm results in accordance with 2013 CDC Guidelines on point-of-care lead testing. If discordant, a 3rd analysis is performed, with the outlier discarded. Prior studies have reported false positive rates of 60-70% for capillary blood lead screening (largely attributed to contamination). As the prevalence of elevated BLLs has been down trending in the U.S., we would expect the false positive rate to rise. Therefore, we were interested in retrospectively determining the false positive rates for the LeadCare Ultra® at our institution, to determine how we compared with published false positive rates. Methods:Performance Insight software (Visiun, Inc.) was utilized to extract results for capillary blood lead screening and confirmatory testing from the laboratory information system (LIS). We extracted data from January 1, 2018 through December 31, 2019 for LeadCare Ultra® and confirmatory testing comparisons received from the reference laboratory (ARUP). Results: There were 83 elevated BLLs out of 14,614 capillary samples tested (overall positivity rate of 0.6%). Of the patients with elevated BLLs, 65 (78%) had a venous confirmatory testing. Excluding samples without confirmatory testing, 31/65 (48%) samples were false positives. Conclusion:At our institution, LeadCare Ultra® had a false positive rate of 48%, which is significantly lower than previously published false positive rates of 60 – 70 % for capillary testing. We hypothesize that our lower false positive rates may be due to implementation of CDC recommendations to confirm all elevated results with a second analysis, and/or the fact that our testing is performed by trained laboratory personnel rather than at the point-of-care. While our false positive rate is an improvement compared to prior published studies, it is still a significantly high rate that could lead to unnecessary alarm for providers and patients. To ensure that providers are aware of the relatively high rate of false positives we have added the following comment to all elevated capillary lead results: "Blood lead levels ≥ 5 ug/dL indicate exposure to lead at levels higher than most individuals. However, 40 - 50% of elevated capillary lead test results are false positives due to skin or environmental contamination of the sample. Therefore, providers should take that into consideration when communicating preliminary results to patients and always confirm elevated capillary blood lead levels with a venous blood sample collected in a trace element tube using a reference lab method." We recommend that clinical laboratories performing capillary lead screening routinely review their retrospective data on false positives and consider appending a comment to educate providers on the high rate of false positives with this testing.

A-366

Evaluation of TSH in a Pregnant Population in a Large Clinical Center in Southern Brazil: An Approach to the Reference Interval

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Introduction: Pregnancy has a profound impact on the thyroid gland and its function. The normal thyroid gland doesn´t show any difficulty in responding to these functional changes during pregnancy, however, when the functional capacity of the gland is compromised, the functioning of the thyroid gland may be compromised; thyroid complications are estimated to affect about 2% of pregnancies. It is extremely important to establish reference intervals for pregnant women for TSH. **Objective**: Establish the TSH reference value through the retrospective data and compare it with results obtained in other studies. **Methods:** Population study, carried out through retrospective analysis of data from a large laboratory in southern Brazil. 17.690 TSH results from pregnant women in the first trimester were evaluated. All samples were collected during the period from 05/2018 to 05/2019, Ultra-Sensitive TSH determinations were performed on the same day of collection using the Advia® Centaur™ analyzer. TSH-related references (LRRs) values outside the limits ("outliers") were removed from Tukey's method. Statistical tests and the estimation of the TSH reference interval were performed using the non-parametric method according to CLSI 28-A3. All patients with one or more of the altered thyroid parameters and TSH above 5mU/L were excluded from the analysis. **Results:** 10% were excluded, the results were presented with the following distribution: 8% less than 0.50U/L, 70% between 0.50 and 3.0U/L, 19% between 3.01 to 4.88U/L and 3% higher than 4.88U/L. The mean age was 26 ± 6.49, reference intervals were evaluated in different age groups: <20, 20 to 30, 30 to 40 and 40 to 50 years, as no differences were observed in the different age groups, we accumulated the results and calculate a single limit. TSH LRR was 0.16 to 4.54U/L. **Conclusion:** The results obtained in this study demonstrate consistency with the other studies , Giavarina D.D 2006[8], in a study of 4800 pregnant women in China, showed that the upper limit of reference was reduced from 5.31 to 4.34 mU/L.; a Meta-Analysis study in China in 2018[10] demonstrated that the reference intervals at the upper limit were close to 4U/L; Nathalie O.[11] in a more recent study in Brazil they had an upper LRR of 3.6U/L. In 2017 the American Thyroid Association (ATA)[6] recommend for VRR pregnancy for TSH according to the local population and, if not possible, adopt a reference interval derived from a similar population. The last recommended alternative is to reduce 0.5U/L from the reference values for non-pregnant women, which would result in approximately 4.30U/L for the test in use TSHUL– ADVIA Centaur Siemens, which is very close of the value obtained in this retrospective study. The lower LRR of TSH obtained in this study was 0.16, 8% of pregnant women had TSH lower than 0.55 mU/L. We understand that the study had some limitations, such as the lack of a clinical evaluation determined according to the inclusion recommendations, being relevant to validate this reference interval through a population study based on the NCBA.

A-367

A 4-Color Multiplexing Digital PCR Assay for Non-Invasive Prenatal Trisomy Testing Using a Novel Digital PCR Platform

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Introduction: Trisomies are the most common fetal aneuploidies with abnormalities in chromosomes 13 (Patau syndrome), 18 (Edwards syndrome) or 21 (Down syndrome). With the discovery of fetal cell-free DNA (cfDNA) and improvements in modern digital PCR technologies, digital PCR has the potential to become an inexpensive and accurate method for trisomy Non-Invasive Prenatal Testing (NIPT) as the standard of care. In this study, we report the first 4-color multiplexing NIPT trisomy test on a novel digital PCR (dPCR) platform to simultaneously detect chromosomes 13, 18, and 21 while using chromosome 1 as the internal reference control.

Methods: The multiplexing assay exploits primers and probes against conserved regions of the chromosomes of interest with 20 sets for each chromosome (Atila BioSystems). The probes were labeled with FAM, HEX, TAMRA and Cy5 fluorophores for chromosome 18, 21, 13 and 1, respectively. We validated the assay on a 5-color integrated digital PCR platform, using eight contrived cfDNA samples that had a range of additional chromosome 21 fetal fractions from 4% to 10% plus a healthy control. The digital PCR platform consists of a micro-molded plastic Microfluidic Array Partitioning (MAP) consumable and a fully integrated single bench-top instrument that combines sample partitioning, thermal cycling, fluorescence image acquisition with control and analysis software. The MAP consumable device has a standard microplate footprint with 16 individual units. Each unit contains 20,000 microchambers with a total of 10 µL loading volume and near-zero dead volume. For the desired accuracy and precision, we pooled 8 units (80 µL total analyzed volume and 160,000 partitions) for each trisomy NIPT sample. Data were analyzed by comparing the ratio of the target PCR concentration to the internal control.

Results: The platform demonstrated that with increased chromosome 21 fetal fraction, the ratio against the chromosome 1 internal control is elevated above one. This 4-color multiplexing trisomy NIPT assay was able to accurately determine the fetal trisomy genotype in all cases on the novel digital PCR instrument. Additionally, this multiplexing digital PCR trisomy NIPT assay can be accomplished within two hours on the integrated platform. **Conclusion**: This is the first 4-color multiplexing trisomy NIPT digital PCR assay developed on a navel digital PCR platform. The platform presented here offers qPCR equivalent workflow, affordability and fast time-to-answer while maintaining high precision and unbiased, absolute chromosome tag quantification for low cost trisomy NIPT.

A-368

A Novel Multimodal Decomposition Statistical Method for Generating Alkaline Phosphatase Reference Intervals in Children: Comparison to CALIPER Project Study Data

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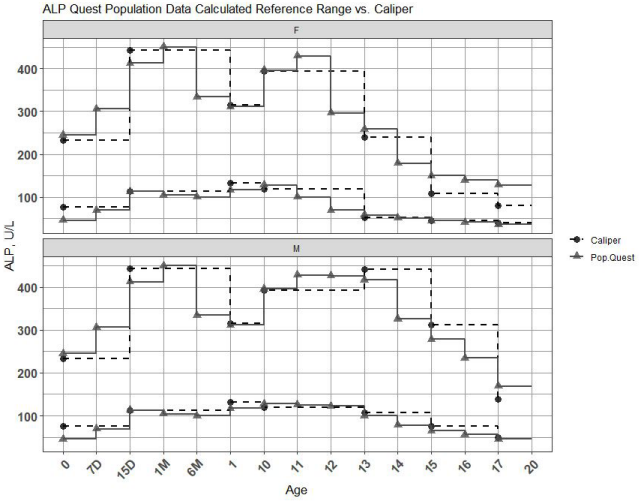
Background: Age exerts a strong influence on alkaline phosphatase because of bone development. Elucidation of pediatric reference intervals is hampered by the paucity of available specimens from healthy children. Data analytics, using patient results, has the ability to identify the healthy subpopulation, and calculate reference intervals for multiple age ranges. We sought to determine whether a novel computerized statistical multimodal decomposition approach using alkaline phosphatase results from a large pediatric patient population database could be used to develop reference intervals. **Methods:** Assuming that multiple subpopulations coexist within patient distribution results, and an adequate number of healthy patients form a subpopulation (multiple modes), a multimodal decomposition in R was used to identify the healthy subpopulation distribution, with a mean and standard deviation (SD). This was applied to a large

retrospective, de-identified population database containing alkaline phosphatase data. Age specific reference intervals were determined using the mean +/- 1.96 x SD. Male and female data were combined up to age 11, then segregated by sex above age 11. Results were internally verified using results from 20 patients at each sex/age interval who were selected based on ICD9/10 screening (Z) codes with further verification by direct comparison to CALIPER reference intervals. Median number analyzed for each proposed sex/age interval was 7,544 (range 2,693 to 1,205,342) with 2.4 million results used.

Results: Internal verification showed that at least 18 of 20 results were within the proposed sex/age reference intervals. External verification using CALIPER reference intervals demonstrated excellent concordance and provided finer detail (Figure). **Conclusion:** The multimodal decomposition method generated pediatric reference intervals comparable to CALIPER reference intervals for alkaline phosphatase. This approach decreases the number of required healthy subjects to establish a reference interval, but it does require adequate-sized data sets and the ability to verify the results with data from healthy subjects.



A-370

Analytical Validation of the Roche Cobas® Elecsys sFlt-1 and PlGF Assays for Diagnosis of Preeclampsia

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Background and objective: Preeclampsia (PE) is a serious complication specific to pregnancy, and one of the leading causes of maternal and fetal morbidity and mortality. PE affects 3 to 5% of pregnancies worldwide. The clinical signs (e.g. hypertension) and currently available laboratory tests (e.g. proteinuria), are neither specific nor sensitive. Therefore, biomarkers for timely and accurate diagnosis of PE are extremely valuable to improve patient outcomes and safety. Recent literature showed that soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are promising serum markers in PE diagnosis. This study aims to examine the analytical performance of sFlt-1 and PlGF immunoassays and their ratio on an automated analytical system.

Methods: The two biomarkers sFlt-1 and PlGF were using Elecsys immunoassay reagents and tested on automated analyzers Cobas® e602 (Roche Diagnostics) in the routine clinical diagnostic laboratory. Precision (reproducibility), linearity, accuracy of the measurement were assessed based on the standard laboratory protocols. For comparison, forty patient serum samples were measured by the Elecsys reagents on Roche e411 analyser (Roche Diagnostics UK) in the laboratory where these assays had been validated through previous clinical studies.

Results: The total imprecision (% CV) of 2.6% and 1.4% was observed at sFlt-1 levels of 102 and 1032 µg/L (Roche PreciControl Multimarker level 1 and 2; n=21, over 12 runs and across two different lots of reagents), respectively. Similarly, the total imprecision of 3.2% and 2.6% was observed at PlGF levels of 99 and 1026 µg/L. Limited by the sample availability, the sFlt-1 method was observed to be linear from 56 to 19393 µg/L, and PlGF was observed to be linear from 14 to 2029 µg/L. In comparison with the assays on e411using independent reagent/calibrator lot, the correlation coefficient (*r*) for sFlt-1 was 0.9998 (*n*=40) with a slope of 1.100 (95% CI: 1.092 to 1.107) and an intercept of -73.8 (95% CI: -106.2 to -41.4), as determined by Deming regression analysis. The correlation coefficient for PlGF was 0.9995 with a slope of 1.029 (1.018 to 1.040) and an intercept of -9.6 (-16.3 to -2.8).

Conclusion: The analytical performance of the assays met the laboratory quality specifications. The comparison study showed an excellent correlation with the previously established methods in an independent laboratory. These assays are analytically validated and ready for clinical validation studies currently underway for the management of suspected preeclampsia.

A-371

Pediatric Reference Intervals for Endocrine Markers and Fertility Hormones on the Siemens Healthineers Atellica IM Analyzer in Healthy Children and Adolescents

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Background: Rapid physiological development in childhood and adolescence combined with lack of immunoassay standardization across manufacturers necessitates the establishment of age-, sex-, and manufacturer-specific reference intervals for endocrine, fertility, and anemia-related immunochemical markers. The present study established age- and sex-specific reference intervals for 11 immunoassays on the Siemens Healthineers Atellica® IM Analyzer in the CALIPER cohort of healthy children and adolescents. **Method:** A total of 600 healthy participants were recruited from the community, and serum samples were collected with informed consent. After sample collection and analysis, age- and sex-specific differences were assessed via the Harris and Boyd method, and outliers were removed from each partition using the Tukey or adjusted Tukey method for normal and skewed distributions, respectively. Reference intervals were then established using the robust method for partitions with 40 to <120 participants and the nonparametric method for partitions with ≥120 participants. **Results:** Of the 11 immunoassays studied, 9 required age partitioning (i.e., DHEAS, estradiol, ferritin, folate, follicle-stimulating hormone, luteinizing hormone, progesterone, testosterone, vitamin B12), and 7 required sex-partitioning. Both free thyroxine and thyroid-stimulating hormone demonstrated no statistically significant age- and/or sex-specific differences. **Conclusion:** Overall, the age- and sex-specific trends observed in this study closely mirror those previously reported by CALIPER and other internationally recognized groups, with most variation occurring within the first year of life and at the onset of puberty. However, established lower and upper reference limits did demonstrate some discrepancies between published values from healthy cohorts on alternate analytical systems (i.e., Roche, Ortho, Abbott, and Beckman), highlighting inter-assay differences between manufacturers and the need for manufacturer-specific reference intervals for pediatric test interpretation.

A-372

Pediatric Reference Intervals for 32 Routine Biochemical Markers on the Siemens Healthineers Atellica CH Analyzer in Healthy Children and Adolescents

M. K. Bohn[1], P. Horn[2], D. League[3], P. E. Steele[2], K. Adeli[1]. *[1]The Hospital for Sick Children, Toronto, ON, Canada, [2]Cincinnati Childrens Hospital, Cincinnati, OH, [3]Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

Background: Accurate and robust pediatric reference intervals are essential for appropriate laboratory test interpretation. With the development of newer analytical systems from manufacturers, *de novo* reference interval establishment is of necessary importance. In the current study, pediatric reference intervals were determined using the robust, and when possible nonparametric, methods for 32 general chemistry analytes on the Siemens Healthineers Atellica® CH Analyzer, with 600 serum samples from the CALIPER cohort of healthy children and adolescents. **Methods:** For all analytes, the need for age and sex partitioning was assessed visually and then confirmed statistically using the Harris and Boyd method. Outliers were then removed from each partition using the Tukey or adjusted Tukey method, depending on data distribution. Following reference interval establishment, results were compared to pediatric reference value distributions previously published by CALIPER for the Siemens Healthineers Dimension Vista®, ADVIA® Chemistry, and Dimension® EXL™ systems. The Dimension Vista general chemistry assays are based on the Dimension assays methodology, while the Atellica CH general chemistry assays are based on ADVIA Chemistry assays, so the transition from the Dimension Vista system to the Atellica CH Analyzers would be expected to produce changes in some pediatric reference ranges,

necessitating module-specific ranges. **Results:** Most analytes had comparable ranges between the two systems; exceptions included Atellica CH Lipase values, which were significantly lower than Dimension Vista values but quite similar to ADVIA Chemistry values. Atellica CH Total and Direct Bilirubin values were higher across all age groups as compared to Dimension Vista values; the difference was largest among the youngest age group's analytes (less than 1 year of age). Atellica CH Calcium and Uric Acid values were slightly higher than Dimension Vista values. **Conclusion:** These data demonstrate the need for module-specific reference intervals for test interpretation in pediatric institutions transitioning from the Dimension Vista system.

A-373

Characterization of Red Blood Cell Fatty Acid Profiles and Age-Dependent Reference Ranges

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Introduction: The health benefits of essential fatty acids (EFA) are well-documented, and deficiencies have been linked to different pathologies, including neurological disorders, heart disease, and poor developmental outcomes. The sequelae of essential fatty acid deficiency (EFAD) are particularly accentuated in infants during periods of rapid growth and development. The current modality for assessing EFAD utilizes plasma levels of EFA, mead acid and the triene/tetraene ratio as indices of EFAD. While these plasma markers have been traditionally used in clinical practice, their levels are influenced by daily changes in dietary intake thus limiting their clinical utility. Due to the estimated 120-day life cycle of red blood cells (RBCs) and slower turnover rate, RBC fatty acids serve as a more accurate marker of long-term nutritional status. RBC fatty acids are less prone to intra-individual variability compared to plasma or serum and do not require fasting, which is unrealistic in pediatric populations. Furthermore, RBC fatty acids reference intervals have never been reported. The objectives of our study were to characterize the RBC fatty acid profiles in pediatric and adult reference populations and to establish age-partitioned reference intervals using a density-based cluster algorithm. **Methods:** RBC fatty acid reference ranges were established using whole blood samples from a population of >400 pediatric and adult controls, age 0 to 62 years, with approval from the University of Utah institutional review board. RBC fatty acids were measured by GC-NCI-MS (Agilent 5977A/7890B) following acid/base hydrolysis and pentafluorobenzyl bromide derivatization. Principal component analysis was used to identify groups of correlated analytes, and a density-based cluster algorithm (R package densityMclust) was implemented for age stratifications. Outliers were removed (according to the Tukey method) from the age groups before partitioning and before undergoing log and Box-Cox transformations to improve normality. The Harris-Boyd method was used to determine reference intervals using R statistical package. **Results:** We characterized RBC profiles for twenty-one fatty acids including omega-3 and omega-6 fatty acids. The analysis revealed significant heterogeneous changes in the concentrations of fatty acids during infancy. There was a 12-fold non-linear decrease in the marker of EFAD, mead acid, changing from 13.9 - 66.7 nmol/mL in the first week of life to 2-5 nmol/mL at age 1 year through adulthood ($p<0.0001$). The triene/tetraene ratio followed similar trend, declining 9-fold from 0.02-0.06 at 0-14 days to 0.0021-0.0061 at > 1 years ($p<0.0001$). In contrast, some fatty acids showed a significant increase during the first year of life. The level of essential omega-6 linoleic acid almost doubled from 160.8 - 422.8 nmol/mL (0-7 days) to 455.7 - 773.2 nmol/mL (> 1 year of age)($p<0.0001$). While changes in several other fatty acids were less pronounced, the concentration of only one out of 21 fatty acids, stearic acid, remained constant across pediatric and adult groups (797-1193 nmol/mL). **Conclusion:** There are significant differences in RBC fatty acid concentrations between pediatric and adult controls. Our established age-dependent reference intervals will be useful to evaluate long-term nutritional status and promptly screen and triage individuals with EFAD for early interventions.

A-374

A Pilot Study: The Relationship between Serum Bisphenol A and Sex Steroid Hormone Levels in Maternal and Child Pairs in a South African Population

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Background: Exposure to Bisphenol A (BPA) during early development particularly in- utero has been linked to a wide range of pathology. Studies have associated BPA exposure to the development of cancers, polycystic ovarian syndrome, pregnancy-related complications and also with cardiovascular disease, obesity and type 2 diabetes mellitus. BPA exerts its varied effects by several different mechanisms. As part of a larger study examining the effect of BPA on maternal and child pairs our aim was to examine the relationship between BPA and its common metabolite BPA- glucuronide (BPA-G) levels and sex steroid hormone levels

Methods: Blood samples and data collected as part of the Mother and Child in the environment (MACE) birth cohort study were utilized for this study. The MACE study population consists of pregnant females recruited from antenatal clinics in Durban, South Africa. Third-trimester serum maternal samples and matching cord blood samples were analyzed for BPA and BPA-g using LC-MS/MS. The same maternal and cord blood pairs were then further analyzed for steroid hormone levels. The following sex steroid hormones were using LC-MS/MS methodology: estradiol, testosterone, 11-deoxycorticosterone, DHEA, androstenedione, 17-OH progesterone, dihydrotestosterone, and progesterone. A commercially available kit the MassChrom Steroid Panel 2 kit (Chromosystems Instruments and Chemicals GmbH, Germany) was utilized for analysis of the sex steroid hormones. Chromatographic separations were carried out using the AB Sciex 4500 triple quadrupole mass spectrometer equipped with an Agilent 1260 ultra-high-performance liquid chromatography (uHPLC) system

Results: Twenty-five maternal and cord blood pairs were analysed for sex steroids and BPA and BPA-g levels. Maternal BPA levels and BPA-G levels median (range) were as follows: 0.95 ng/mL (0.4- 15.3) and 4.71 ng/mL (0.48-21.8). Median cord BPA levels 0.92 ng/mL (0.4-13.2) and BPA-G levels 4.21 ng/mL (0.4-26). Median maternal and cord blood estradiol levels were respectively 118 nmol/l (range 4-772) and 67 nmol/L (range 3-371). Median maternal and cord blood testosterone median(range) levels were respectively 2.3 nmol/L (0.34-17.7) and 0.66 nmol/L (0.16-14.9). Spearmans correlation for maternal BPA and BPA-G levels with cord estradiol were as follows ($r=0.3$;$r=0.25$) and cord testosterone levels ($r=0.4$; $r=0.04$) , respectively.

Conclusion: The findings suggest that there is a possible effect of BPA on steroid hormones however in this pilot cohort it was not substantial.

A-375

Urine Specimens Collected via Cotton Balls: Can They Be used for Pediatric Patient Testing?

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Introduction Urinalysis is not only used to assess the urinary system functions but also to assist in the diagnosis of certain diseases. Urine samples are usually directly collected in clean, sterile containers to prevent contamination or interference. An alternative urine collection method such as collecting via cotton balls is used when the direct collection is challenging. The technique is especially useful for neonates and young children. However, the literature evaluating the effects of cotton balls on the accuracy of test results is limited. The aim of this study was to investigate the validity of laboratory results from urine samples collected via cotton balls as compared to paired samples collected directly in urine collection cups. The tests selected were based on the clinical importance for pediatric hematology and oncology services. **Method** Forty-one patient samples used in this study were collected and de-identified after all the clinically ordered tests were completed. Samples were collected directly in clean collection cups and stored under refrigeration before and after the ordered tests were performed. To mimic urine collection via cotton balls, for each sample, 10 mL of urine was poured onto a pile of five cotton balls (Tidi Product, Neenah, WI). After 30 mins, the urine was recovered from the cotton balls using a syringe. Bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrite, and leukocytes were semi-quantitated using vChem 10sg dipsticks and Multistic 8sg

dipsticks. Specific gravity was measured by a refractometer. The concentration of creatinine, sodium, potassium, chloride, and total protein were measured on a Beckman Coulter DXC 600 analyzer. Microscopic examinations were also conducted. Statistical analyses were performed using the R program. The categorical data were analyzed using Fisher's exact test, and the numerical data were analyzed using the Wilcoxon signed-rank test. The difference in the numerical results of each analyte was compared with its corresponding reference change value (RCV). If the mean difference % of an analyte was within its RCV %, the difference is not considered clinically significant. **Result and Conclusion** Urine collection via cotton balls did not cause a significant change in bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrite, leukocytes, and specific gravity ($p>0.1573$). Creatinine, sodium, potassium, and chloride concentrations were significantly different statistically ($P<0.0001$); however, the differences were not clinically significant. The mean difference of creatinine, sodium, potassium and chloride was 2.00 ± 2.00 %, 2.20 ± 3.40%, 1.86 ± 1.77%, 1.00 ± 2.00%, respectively. The microscopic examination results of crystals, blood cells, epithelial cells, and casts by microscopic examination were significantly altered in specimens with cotton balls. The results suggest that urine collection via cotton balls is suitable for routine urinalysis, while the microscopic examination of such urine samples is not acceptable.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Clinical & Diagnostic Immunology

B-006

Seeing Double: MASS-FIX Characterization of Immunofixation Bands as Monoclonal or Biclonal

E. M. Fatica, M. A. Martinez, P. M. Ladwig, J. Murray, T. Kourelis, A. Dispenzieri, J. A. Lust, M. R. Snyder, R. A. Kyle, D. L. Murray, M. A. V. Willrich. *Mayo Clinic, Rochester, MN*

Background: Plasma cell disorders are typically characterized by excessive production of a single monoclonal protein (M-protein). However, a small subset of patients have more than one identifiable M-protein. Isotyping of M-proteins has traditionally been done by gel-based immunofixation electrophoresis (IFE). However, it is not possible to elucidate from the IFE gel if the presence of two bands of the same isotype represent two unique clones, monomers and dimers of a single clone, or one clone with post-translational modifications. A novel assay using immunoenrichment coupled to matrix-assisted laser desorption ionization time-of-flight mass-spectrometry (MASS-FIX) for detection and isotyping of M-proteins was developed to replace IFE. The aim of this study was to use the MASS-FIX assay to characterize two bands of the same isotype as monoclonal or biclonal.

Methods: Patient samples with two bands of the same isotype identified by IFE were collected for further analysis. Samples were enriched using nanobodies against IgG, IgA, IgM, or κ and λ light chains, then analyzed as reported previously using either MALDI-TOF (MASS-FIX) (PMID: 27540026) or LC-ESI-TOF (PMID: 25916620) mass spectrometry. Light chain masses were used to differentiate IgGκ M-proteins from therapeutic monoclonal antibodies (T-mAb) (+1 charge state; *m/z* 23380 for daratumumab, 23423 for elotuzumab, and 23488 for isatuximab). Mass shifts between M-protein peaks obtained via MASS-FIX were calculated. Mass differences of 72±3Da or 162±2Da were attributed to matrix adducts or glycosylation, respectively. Patients were identified as having biclonal M-proteins if mass shifts between peaks did not correspond with these mass differences.

Results: 85 residual patient samples were collected, including 37 IgG, 27 IgA, and 21 IgM M-proteins. 93.3% (n=25) of IgA samples were identified as monoclonal, indicating the presence of monomers and dimers of a single clone on the IFE gel. 40.5% (n=15) of IgG samples were truly biclonal and 59.4% (n=22) were monoclonal, of which 10.8% (n=4) were glycosylated, a notable observation as patients with glycosylated light chains are at higher risk for AL amyloidosis. Within the monoclonal IgGs, 26.6% (n=5) of the IgGκ's had masses consistent with the presence of T-mAbs in addition to an IgGκ clone. 42.8% (n=9) of IgM samples were biclonal and 57.1% (n=12) were monoclonal, of which 9.5% (n=2) were glycosylated. MASS-FIX sample data was compared to high resolution LC-ESI-TOF sample data in a subset of samples. Classification of biclonal M-proteins as well as identification of T-mAbs and glycosylation patterns agreed between the two methodologies in 22/22 cases, suggesting that MASS-FIX has sufficient resolution to distinguish monomers and dimers of M-proteins. There was a significant difference in the prevalence of monoclonal versus biclonal M-proteins for IgA when compared to IgG and IgM (Chi square 10.04, p=0.00657).

Conclusion: Here we demonstrate the utility of mass spectrometry in the characterization of multiple IFE bands of the same isotype. IgAs are most commonly seen in monomeric and dimeric forms, while IgG and IgM have a higher incidence of biclonal M-proteins. Improved reporting accuracy of M-protein clones may be useful in prognosis or monitoring of patients with plasma cell disorders.

B-009

Establishing Healthy Distribution for Thyrotropin Receptor Antibodies, Thyroid Stimulating Immunoglobulin and Thyroid Stimulating Blocking Antibody for Individuals in Beijing, China

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Background: Autoimmune thyroid disease involves thyrotropin receptor antibodies (TRAb), thyroid stimulating immunoglobulin (TSI) and thyroid stimulating blocking antibody (TSBAb), which are of great value in the diagnosis of disease. However, the distribution of the three antibodies in the health individuals has not been established. This study will establish a healthy distribution of the three antibodies in the Chinese population with suitable methods to provide theory basis for diagnosis of autoimmune thyroid disease.

Methods: In total, 120 apparently healthy individuals were included in this study by questionnaire. Thyrotropin binding inhibitor immunoglobulin (TBII) assay was used for the measurements of TR-Ab, IMMULITE 2000 TSI assay for TSI, and enzyme linked immune sorbent assay (ELISA) for TSB-Ab.

Results: The baseline level of common biochemical analytes of individuals enrolled in the study were in the normal range. The distribution of TR-Ab in males was significantly higher than that in females ($P<0.05$), while there was no statistical difference for TSB-Ab measurements between males and females. The healthy distribution of TRAb, TSI and TSBAb were established.

Conclusion: TRAb and TSBAb are dispersed in healthy individuals, while TSI cannot be quantified in healthy individuals due to methodological reasons. We suggest that TSBAb and TRAb health distribution be taken into account when diagnosing autoimmune thyroid disease.

B-010

Regulation of Pathogenic Th17 Cells Differentiation by miR-124-3p/YY1 Axis in Rheumatoid Arthritis

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Background: Our previous studies have revealed that transcription factor YY1 played an important role in the pathogenesis of rheumatoid arthritis (RA).Moreover, studies showed that the pathogenic TH17 cells involved in RA pathogenesis. However, the regulatory mechanism of YY1 in RA and whether it has any role in the differentiation of pathogenic TH17 cells remain unclear. The present study aims to investigate the role of YY1 in differentiation of pathogenic TH17 cells and its possible regulatory mechanism in RA.

Methods: Peripheral blood of was obtained from patients with RA and healthy donor (HD) and the proportion of pathogenic TH17 cells was detected by flow cytometry, and the clinical and laboratory data were collected. Pathogenic TH17 cell differentiation system was constructed in vitro. YY1 knockdown was performed by YY1 shRNA lentivirus to investigate the role of YY1 in the differentiation of pathogenic Th17 cells. miRNAs which could bind to the 3 'UTR region of YY1 were predicted by TargetScan and validated by dual-luciferase reporter system.

Results: The proportion and number of pathogenic Th17 cells in peripheral blood of RA patients were significantly higher than that of HD group. In vitro, the differentiation of pathogenic TH17 cells was inhibited by YY1 knockdown. miR-124-3p could bind to 3'UTR resign of YY1 to inhibit the posttranscriptional translation of YY1.A negative correlation between the expression of miR-124-3p and YY1 was found.

Conclusion: The present study demonstrated that the miR-124-3p/YY1 pathway could promote the differentiation of pathogenic TH17 cells which involved in the inflammation process of RA. Targeting of miR-124-3p and YY1 may be a novel therapeutic strategy for RA.

B-011

The Impact of Handling and Long-Term Sample Storage on the Assessment of Functional Complement using the Binding Site CH50 Assay on the Optilite Analyser

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Background: Functional assessment of complement activity is used in the screening and monitoring of immunological diseases such as systemic lupus erythematosus, and other acquired or inherited complement deficiencies. Careful consideration must be taken with sample handling and storage, as complement activation can occur *ex-vivo* leading to false results. Here we report the stability of CH50 activity after different sample storage conditions, measured by the CH50 liposome assay developed for use on the Optilite® analyser.

Methods: Serum samples from healthy adult donors were used (n=59-61, self-certification absence of autoimmune conditions, fever or respiratory illness). CH50 activity was determined using the Optilite CH50 assay (The Binding Site Group Ltd, UK). 6 replicates of the d0 sample were taken, with subsequent measurements in duplicate following storage at room temperature, 4°C, -20°C or -80°C. Stability was also as-

sessed following sequential freeze/thaw (FT) cycles with storage at -20°C or -80°C. Sample stability was assessed utilising a method based on CLSI guideline EP25-A with measurand drift used as the primary metric and an allowable error of +/-15%.

Results: The median value for samples at d0 was 65.17 U/mL (range 35.17 to 93.82 U/mL), with a median within-sample CV of 0.99% (range 0.18 to 6.2%). After 1d at RT there was a median 17% decrease in CH50 activity (range -33 to +2%; p<0.0001). At 4°C the change in CH50 activity ranged from a median 9% decrease (range -44 to +3%; p<0.0001) after 1 day, to a 21% decrease (-68 to +2% p<0.0001) after 7 days. Following longer term storage at -20°C a time dependant decrease in CH50 activity was observed (median percentage change at 7days -13%, range -20 to +5%, p<0.0001; 1 month -19%, range -29 to -4%, p<0.0001; 6 months -41%, range -59 to -22%, p<0.0001). The time dependant decrease in CH50 activity seen with storage at RT, 4°C and -20°C was not observed with -80°C storage for up to 1 year (median percentage change at 7days -10%, range -23 to +8%, p<0.0001; 1 month -5%, range -23 to +11%, p<0.01; 6 months +5%, range -26 to -20%, p<0.01; 1 year 1%, range -16 to +18%, ns). The stability time point for the different storage temperatures were; 1 day at RT, 5 days at 4°C, 42 days at -20°C and 389 days at -80°C. Where samples were subjected to sequential FT cycles, a FT cycle dependent decrease in CH50 activity was observed. The median decrease in activity after 1 FT with -20°C storage was 9% (range -22 to +1%; p<0.0001) and with -80°C storage a median 5% decrease was observed (-21 to +4%; p<0.0001). Following 5 sequential FT cycles there was a 25% decrease (-68 to -7%; p<0.0001) in activity with -20°C storage, and a 9% decrease (-66 to +13% p<0.0001) with -80°C storage. **Conclusion:** Sample storage and handling can have a significant impact on functional complement assessments. If possible, room temperature storage should be avoided and where long-term storage is required, storage at -80°C is optimal to preserve complement function.

B-012

Preliminary Performance of Access hsTnI, PCT, and TSH 3rd IS Assays on a Next-generation Prototype Analyzer†

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Background: Beckman Coulter's next-generation, high-volume immunoassay analyzer is currently under development. The analyzer is designed to achieve faster throughput, employ an enhanced chemiluminescent substrate (LumiFAST), deliver shorter turnaround times, run more tests per reagent pack, and provide improved user workflows, when compared to the legacy Access UniCel DxI 800 Immunoassay System. The development has focused on system improvements, but the bottom-line goal is to ensure high-quality patient results. Assay data generated on the prototype analyzer demonstrates strong correlation and similar imprecision to the predicate platform, the Access 2 Immunoassay System. Highlighted here are select assays which are demonstrating improvements in sensitivity, like thyroid stimulating hormone (TSH), troponin (hsTnI), and procalcitonin (PCT). **Methods**: A comprehensive assay menu has been characterized on the prototype analyzer and all data is compared to predicate platform performance. A panel of residual samples spanning the assay analytical measuring ranges and commercially available quality controls were tested across three reagent lots, two calibrator lots, multiple days of testing on three prototype analyzers and on three Access 2 systems. Accuracy, imprecision, and sensitivity were calculated and compared to the Access 2 predicate. Additional performance criteria have also been evaluated to understand assay linearity and dilution recovery. **Results**: A subset of preliminary data for the Access TSH 3rd IS, Access hsTnI, and Access PCT assays generated on the prototype analyzer is presented here. Each of these assays exhibit a strong correlation to Access 2, similar imprecision, and improved sensitivity as measured by limit of quantitation (LoQ). Further, both the TSH and PCT assays have more tests available from each individual reagent pack. **Accuracy** (Passing Bablok slope, A2 vs. Prototype analyzer†): TSH 200 tests/pack (0.999), hsTnI (1.00), PCT 100 tests/pack (1.03). **Variance** (Access 2, Prototype analyzer†): TSH (6.6%, 5.9%), hsTnI (8.7%, 6.2%)*, PCT 100 tests/pack (6.4%, 4.3%). **Sensitivity** (Access 2, Prototype analyzer†): TSH 200 tests/pack (20% CV LoQ calculated surrogate: 0.005 µIU/mL, 0.002 µIU/mL), hsTnI (10% CV LoQ calculated surrogate: 2.9 pg/mL, 0.76 pg/mL), PCT 100 tests/pack (20% CV LoQ calculated surrogate: 0.005 ng/mL, 0.003ng/mL).

*Includes all samples above LoQ

† The prototype is not cleared or approved for commercial use is any geography. **Conclusions**: Preliminary assay data generated on the next-generation, high-volume immunoassay analyzer prototype system demonstrates correlation to Access 2 and enhanced performance. Additionally, characterization data demonstrates the opportunity to achieve improved sensitivity for assays including Access TSH 3rd IS, Access hsTnI, and Access PCT.

B-014

Use of the Binding Site and Diazyme Serum Free Light Chain Assays in Patients with Monoclonal Gammopathies and Healthy Populations

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Background: The analysis of free light chains in serum is now standard of care in both the screening, diagnosis and monitoring of patients with or suspected of having multiple myelomand other monoclonal gammopathies. Until recently, the only assay available was the Freelite™ assay (The Binding Site Group, Birmingham, UK). However, a new immunoassay to quantitate free kappa and free lambda assay has become available from Diazyme, offering clinical laboratories a choice in reagents. The objectives of our study are the following: 1) To analytically validate the Diazyme free light chain assay for potential clinical use 2) To determine clinical interchangeability of the two assays in both patients with monoclonal gammopathies as well as a presumed healthy population **Methods:** The Diazyme kappa and lambda light chain assays were placed on the Abbott Architect c8000using parameters obtained from the assay manufacturer package insert. Precision and linearity were assessed using material provided by the manufacturer. Accuracy and reference ranges were assessed using patient serum specimens. Waste serum specimens from 61 patients who had SPEP/IFE/Freelite testing performed in the clinical laboratory were obtained. In addition, serum from a total of 127 presumed healthydonors was collected. Free light chains were quantitated for these specimens on the Abbott Architect c8000 using Diazyme assay kits as well as on the Binding Site Optiliteusing Freelite reagents. **Results:** 12 of the 61 myeloma patient specimens could not be compared due to missing kappa or lambda values from the Diazyme assay. Missing values were due to an error message from the Abbott c8000 due to kappa/lambda values exceeding the analytical measurement range (AMR). Specimens that resulted in errors on the Diazyme assay were overwhelmingly patients with free light chain disease. A comparison of the 49 specimens that could be analyzed by the Diazyme assay with the Binding Site Freelite assay showed significant negative proportional bias noted at high concentrations of both kappa and lambda light chains. Within this cohort, 30 out of 49 had abnormal kappa/lambda ratios using Freelite, while 37 had abnormal kappa/lambda ratios that fell outside the Diazyme suggested k/l ratio range. In the cohort of 127 presumed healthy donors, 9 were classified as having an abnormal k/l ratio based on Freelite results, while 106 were classified as having an abnormal k/l ratio based on the Diazyme assay results. The 95% interval for the kappa/lambda ratio based on the Freelite assay was 0.66-1.82, while the ratio based on the Diazyme assay was 1.1-9.96 for this population. **Conclusion:** Use of the Diazyme assay will require the laboratory to develop their own dilution protocols to analyze specimens with elevated kappa or lambda levels. Further dilution may be required even when the instrument gives a numerical result, as the resulting number may potentially be falsely low. There is also significant discordance in K/L ratios between the Binding Site and Diazyme assays both in patients and healthy donors. For this reason, current medical decision points based on K/L ratios will have to be re-determined in separate studies for the Diazyme assay.

B-015

Evaluation of Basic Performance of Lumipulse G SCC Assay for LUMIPULSE® G Systems

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Background: The SCC antigen (squamous cell carcinoma-related antigen) measurement reagent is used as a diagnostic aid for squamous cell carcinoma of various organs such as cervical squamous cell carcinoma, endometrial carcinoma, esophagus cancer, skin cancer, head and neck cancer and lung squamous cell carcinoma. Recently we developed new SCC IVD kit, which is fully automated chemiluminescence enzyme immunoassay (CLEIA) for LUMIPULSE G systems (LUMIPULSE G1200 and LUMIPULSE G600II) for the quantitative measurement of SCC. In this study, the basic performance of the new Lumipulse G SCC assay was evaluated.

Methods: Lumipulse G SCC is a two-step sandwich CLEIA. The resulting reaction signals are proportional to the amount of SCC in the serum or plasma sample allowing quantitative determination of SCC. Analytical performance of the assay was evaluated on LUMIPULSE G1200 according CLSI guidelines.

Results: 1)Sensitivity:Limit of detection (LoD) and quantitation (LoQ) were ≤ 0.03 ng/mL and ≤ 0.04 ng/mL, respectively. 2)Linearity:Linearity was demonstrated over the range 0.06 - 197.42 ng/mL. 3) Reproducibility:Total precision was ≤ 3.8%. 4) Interference by biological materials:The measurement value variations by various biological materials (bilirubin, hemoglobin, triglycerides, chyle, total protein, rheumatoid factor and HAMA) were ≤ 9% at the clinically high enough concentration.

5) Method comparison:Results of method comparisons were correlation coefficient = 1.0 regression slope = 1.1 against ARCHITECT SCC, correlation coefficient = 1.0, regression slope = 1.0 against Elecsys SCC, and correlation coefficient =1.0, regression slope = 1.1 against Lumipulse Presto SCC which is another Lumipulse product line (sold only in Japan). 6) Reactivity : Lumipulse G SCC detected not only SCCA1 antigen but also SCCA2 antigen.

Conclusion: Lumipulse G SCC assay showed good analytical performances. Especially, it has the highest level of sensitivity and the widest measuring range compared with other predicate devices. It is expected that the new assay is useful as an aid in the diagnostics and monitoring of squamous cell carcinoma patients.

B-016

High Sensitivity Troponin I Characterization on Alinity vs. Architect at Two Unique Sites

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Background: TnI is the biomarker of choice when evaluating acute coronary syndrome (ACS) and cardiac disease. The utility of this biomarker in early detection of myocardial infarction (MI) and risk stratification of cardiac disease relies on the assay's performance at the 99th percentile cut-points established for the asymptomatic populations. The IFCC cardiac task force has set out specific guidelines covering the definition of a high sensitivity troponin assay. One requirement is the capability of the assay to detect at least 50% of the healthy population above the claimed limit of detection (LOD). This highlights the increased usage of the hsTnI assay as a tool for cardiac disease monitoring and treatment while continuing to serve as an ACS and MI diagnostic marker.

Objective: The purpose of this study was to characterize the Alinity hsTnI assay in comparison to its predicate ARCHITECT hsTnI in both asymptomatic and hospital populations. Results for the hs-TnI on Alinity compared to the ARCHITECT predicate assay at two distinct sites.

Methods: A total of 1000 samples from healthy donors were evaluated. Samples were screened using Natriuretic peptides (NT-proBNP/BNP), fasting Glucose and Creatinine concentrations to rule out co-morbidities that would implicate potential cardiac disease. The 99th percentile of the normal male and female populations were determined for both assays. The sites also performed independent method comparisons on 234 residual samples from hospital patients and 300 specimens from asymptomatic healthy individuals. Finally, the sites evaluated 5-day CLSI precision on the hsTnI to verify performance.

Results: The observed results for precision met expectations with < 6%CV. Method comparisons for the assays are shown in the table below along with non-parametric 99th percentile calculations overall and by sex as determined for this study.

Conclusion: The Alinity hsTnI assay performs as expected and compares extremely well to the ARCHITECT results in both the asymptomatic and hospital populations.

Method Comparison and 99%ILE Calculation Table				
SITE	N	RANGE(ng/L)	SLOPE	R^2
UHN	234	1-30	0.90	0.95
UHN	200	1-15	0.92	0.93
UCD	300	1-37	1.05	0.98
UCD	N	SEX	ARCHITECT 99%ILE	Alinity 99%ILE
	457	Female	8.7 ng/L	8.8 ng/L
	468	Male	29.6 ng/L	30.0 ng/L
	925	All	14.6 ng/L	15.0 ng/L

B-018

Identification of DAGLA as an Autoantibody Target in Cerebellar or Hippocampal Degeneration

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Background: Autoantibodies against Purkinje cell antigens are frequently connected with cerebellar ataxia. We identified diacylglycerol lipase alpha (DAGLA) as novel Purkinje cell antigen targeted by sera of twelve patients with similar IgG reactivity on cerebellar tissue. One patient with clinical data available presented with cerebellitis (P11), the other with epilepsy and hippocampal sclerosis (P12).

Methods: Sera of five patients suffering from neurological aberration (P1 - P5) were subjected to comprehensive autoantibody screening by indirect immunofluorescence assay (IFA) and immunoblot. Immunoprecipitation with lysates of cerebellum followed by mass spectrometry was used to identify the autoantigen. Antigen identification was verified by Western blot and IFA with a monospecific rabbit antibody against the respective target antigen and in several immunoassays with the recombinant protein expressed in HEK293 cells. Furthermore, sera with a similar staining pattern as P1 to P5 without known autoantibody reactivity (n = 115), as well as negative control sera without a specific reaction in IFA with neuronal tissues (n = 51), were screened for anti-DAGLA antibodies. For epitope mapping, six different fragments of the DAGLA protein were recombinantly expressed in E. coli, purified and analysed in ELISA using anti-DAGLA positive patients' sera and healthy controls.

Results: IFA screening of sera from P1 to P5 revealed IgG reactivity with the molecular layer in rat and monkey cerebellum. The dendrites of the Purkinje cells were stained, whereas the Purkinje cell somata did not react. Furthermore, no IgG reactivity was found with a panel of 30 recombinantly expressed established neural autoantigens. The sera of P1 to P5 immunoprecipitated Sn1-specific diacylglycerol lipase alpha (DAGLA), as detected by Coomassie-stained SDS-PAGE followed by mass spectrometry or by Western blot using a monospecific rabbit antibody against DAGLA. Immunolabelling of cerebellar sections of rat and monkey brain with the rabbit anti-DAGLA antibody revealed the same staining pattern as the patients' sera. Anti-DAGLA reactivity of the sera of P1 to P5, as well as seven additional sera (P6 to P12) with a similar staining pattern on cerebellum, was confirmed by IFA with the recombinant protein. Sera from healthy controls were all negative. In a neutralization experiment, recombinant DAGLA abolished the tissue reactivity of the samples, thus verifying the correct antigen identification. ELISA using recombinantly expressed fragments of the human DAGLA protein revealed the main reactivity of patients' sera with the c-terminal intracellular fragment.

Conclusion: DAGLA is a cell membrane protein predominantly expressed on the dendritic surface of Purkinje cells and in hippocampal pyramidal cells. By catalysing the synthesis of 2-arachidonoylglycerol, the most abundant endocannabinoid in tissues, DAGLA influences synaptic signalling. Antibodies against DAGLA represent novel biomarkers for the diagnosis of neurological autoimmune diseases associated with cerebellar or hippocampal degeneration.

B-022

Testing for Specific Serum IgA in Addition to IgM has An Added Value in Acute Secondary Dengue Virus Infections

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Background: Dengue virus (DENV) is a widely distributed flavivirus. Its serodiagnosis based on antibody detection is affected by cross-reactivity against other flaviviruses. In acutely DENV-infected patients who contracted the virus as a secondary DENV/flavivirus infection, the IgM response to DENV can be low or undetectable, while IgG presents early and at high titers. To determine the usefulness of IgA in the serodiagnosis of acute DENV infections, its diagnostic performance was compared to that of IgM in endemic and non-endemic settings.

Methods: Panel A consisted of serum samples from 20 Thai patients. Each patient gave three sequential samples between day one and 18 after symptom onset (60 samples in total). All patients suffered from an acute secondary DENV/flavivirus infection as classified by positive testing for DENV nucleic acid using PCR, by and high

initial IgG titers. Panel B consisted of 72 serum samples from 33 travellers returning from non-endemic regions with acute DENV infection (confirmed by PCR or antigen detection). These samples were obtained sequentially until day 196 after symptom onset. Additionally, we examined sera from 250 healthy German blood donors and 51 patients with acute West-Nile virus (WNV) or Zika virus (ZIKV) infection to evaluate specificity. Samples were analysed for anti-DENV IgM and IgA antibodies using Anti-DENV ELISAs (EUROIMMUN, Germany) based on purified DENV particles.

Results: Until day ten, sensitivities for anti-DENV antibodies were higher for IgA compared to IgM in panel A. After day ten, combined testing for IgM and IgA resulted in a high sensitivity of 95.2%, that outperformed the sensitivity of testing for IgM or IgA alone. Panel B showed comparable IgM and IgA reactivities throughout the infection. The ELISAs yielded identical specificities in blood donors (98.4%) and WNV-infected patients (25%), but the IgM ELISA had a higher specificity (90.9%) compared to that of IgA (81.8%) in ZIKV-infected patients.

Conclusion: Testing for anti-Dengue IgA antibodies is advantageous as it results in increased sensitivities, especially in the early phase of secondary infection, where the Anti-Dengue ELISA (IgA) closes the diagnostic gap in case of low/undetectable IgM. However, testing for IgA provides no additional value for serodiagnostics of primary DENV infections.

B-023

Time-Related Performance Characteristics of High-Sensitivity Troponin I Controls and Reagents

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Background: The clinical usage of high sensitivity Troponin (hsTnI) testing has evolved beyond the uses for patients with suspected ACS to the more challenging use of this biomarker in risk stratification of asymptomatic patients and apparently healthy individuals.The advent of these new applications for the Troponin test drives an increased need for careful assessment of quality control. These quality control measures must span over more extended periods of time and larger numbers of reagent lots. Reagent lot to lot consistency is critical to ensuring accurate and timely reporting of results to both the physicians and patients involved. The present study aims to do a look back over several years, instrument systems, and reagent and control lots in our laboratory (St Vincent's Hospital) to evaluate variance factors and their potential impact on overall hsTnI measurements. The evaluation also obtained release data from the manufacturer over the same time period for comparison purposes.

Methods: Imprecision (%CV), bias, intra- and inter-lot variability of hsTnI QC and reagent materials were evaluated using hsTnI assay data collected between 2015 and 2019 on three different Abbott instruments: Architect CI4100, Architect I2000SR and ALINITY CI (Abbott Diagnostics, Abbott Park, IL). Testing were performed in the Core Lab Facility based in UCD Clinical Research Centre St.Vincent's University Hospital, Dublin Ireland.Bias and intra- and inter-lot CV was calculated for controls and assays. The effect of control and assay age on the hsTnI measurements was analysed using linear models.

Results: During the five years, 32 reagent lots and 18 QC lots were used at UCD. For the same period the manufacturer tested 305 reagent lots. The imprecision for all control values for all reagents was less than 10% CV.

Conclusion: The age of controls and reagents had minimal effect on bias. Multiple reagent and control lots showed minimal impact on lot to lot variability over a fairly long period of time (5 years).

B-024

Stool Extraction by Manual Weighing Versus Device for Fecal Calprotectin: What's the Difference?

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Objective: Inflammatory bowel disease (IBD) is a disorder, which causes chronic inflammation of the digestive tract. The most common types of IBD are ulcerative colitis and Crohn's disease. Fecal calprotectin (FC) is a non-invasive screening test that is sensitive and specific for intestinal inflammation; it helps distinguish IBD from disorders that present with similar clinical symptoms, but lack an inflammatory component. FC testing is increasing, and therefore ease of testing is an important consideration. One bottleneck for FC testing is the extraction step, as each stool specimen has to be accurately manually weighed using an analytical balance. However, newer FC assays have FDA-approved stool extraction devices that streamline the extraction process. The devices are pre-packaged tubes that contain a stick with grooves, and the manufacturers claim that the grooves accurately and precisely collect a certain amount of stool for the extraction (10-50 mg). Therefore, the aim of this study was to evaluate the accuracy and precision of extraction by device compared to manual weighing. **Methods:** We evaluated the performance of two FC assays with extraction devices, DiaSorin LIAISON Calprotectin chemiluminescent immunoassay and INOVA QUANTA Lite Calprotectin ELISA. First, comparisons by Deming regressions and % biases of extraction by device versus manually weighing were examined for the LIAISON and INOVA assays using 32 stool samples (Bristol stool types 2-6). Second, three stool samples were selected to evaluate the pre-analytical precision of device-extracted results at a low, medium, and high calprotectin concentration. For precision, two technologists extracted each sample in duplicate by manually weighing and device for a total of eight technical replicates per sample. **Results:** Comparison by Deming regression of extraction by device versus manually weighing for LIAISON and INOVA had slopes of (1.741 and 1.031) and biases of (28% and -7%), respectively. One elevated calprotectin specimen had an 85% difference between manually weighing (2,040 µg/g) versus the device (3,780 µg/g) for LIAISON. Therefore, we excluded this result, and the slope was 1.126 with a bias of 0.9% for LIAISON. The average, absolute percent difference between manual weighing compared to device was 10% for LIAISON and 18% for INOVA. However, over half of all stool samples had higher results when specimens were extracted by manually weighing versus device, suggesting that the devices slightly under recovered. For the pre-analytical precision of the extraction by manual weighing versus device, the standard deviations were comparable across most of the replicates. Our data did not suggest that the device had more imprecision than the current manual weighing process; however, the technologists had to ensure that the extraction grooves of the device were properly filled with stool for each sample. **Conclusions:** Overall, the new extraction devices had acceptable performance when compared to manually weighing for samples that are within Bristol stool types 2-6. Since laboratories that perform FC testing will likely need to use both extraction processes for different types of stool samples, it is important to ensure that the accuracy and precision of both extraction methods are comparable for consistency of FC results.

B-027

TGF-β1 and TNF-α: Antagonistic Cytokines Controlling Extracellular Matrix Remodeling after Radioactive Iodine Therapy for Papillary Thyroid Carcinoma

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Background: Radiotherapy has a significant effect on the modulation of immune responses, and this effect is mainly due to the production of cytokines by the tumor cells. The total thyroidectomy, followed by radioactive iodine (I-131) ablation of the post-surgical thyroid remnants, is considered the ideal treatment for papillary thyroid carcinomas (PTC) with/without Hashimoto's thyroiditis (HT). We aimed to assess the effects of therapeutic irradiation with I-131 on the production of transforming growth factor-beta 1 (TGF-β1), tumor necrosis factor-alpha (TNF-α) and its soluble receptors sTNFRI, sTNFRII, matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1), in PTC with/without HT patients.

Methods: Fifty-four patients with PTC (8M/46F) and 41 with PTC+HT (3M/38F) were investigated. PTC+HT patients had positive titers of anti-thyroglobulin antibodies (TgAb). Peripheric blood samples were collected before and four days after I-131 administration. All patients received the same dose of I-131 (3.7 GBq). The serum levels of TgAb, TGF-β1, TNF-α, sTNFRI, sTNFRII, MMP-9, and TIMP-1 were measured by ELISA. Statistical analysis was conducted using Statistica 8.0. Significance was set at a P-value < 0.05.

Results: I-131 therapy of PTC+HT patients was associated with an increase in the TgAb level (P=0.001), TGF-β1 (P=0.001), MMP-9/TIMP-1 ratio (P=0.06) and a decrease in TNF-α/sTNFRI, TNF-α/sTNFRII ratios (P<0.001). TgAb titers were positively related to MMP-9/TIMP-1 ratio (r=0.52, P<0.01). In the PTC group, the beneficial effect of I-131 was illustrated by a significant reduction of TGF-β1 (P=0.001), MMP-9/TIMP-1 ratio (P=0.003), and an increase in TNF-α/sTNFRI, TNF-α/sTNFRII ratios (P<0.001). The reduction of the MMP-9/TIMP-1 ratio was positively correlated with TNF-α level at follow-up (r=0.67, P=0.009). **Conclusion:** Elevated TNF-α/sTNFRI and TNF-α/sTNFRII ratios indicate a decline in disease activity after I-131 therapy, more pronounced in PTC than in PTC+HT. This suggests that suppression

of sTNFRI, sTNFRII, or increased production of TNF-α is required to reduce extracellular matrix deposition and to initiate remission of cancer. Our results show the functionally antagonistic nature of TNF-α and TGF-β1. This finding represents a useful paradigm to study the complex cellular signals that regulate extracellular matrix remodeling after I-131 therapy in PTC with/without HT patients.

B-030

Diagnostic Value of Neurofilament Light Chain in Multiple Sclerosis and Other Central Nervous System Diseases

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Background: Multiple Sclerosis(MS) is a chronic, neuroinflammatory , neurodegenerative central nervous system(CNS) disease. This disease is one of the most common causes of physical and cognitive disability in young adults. There is still no clinically and analytically validated biomarker for the diagnosis, follow-up, and response to the treatment of MS. Neurofilament light chain(NFL) is one of the candidate biomarkers for MS. It is the main components of the axonal cytoskeleton and this protein is an exclusive product of neuronal cells. Hence, their release into the extracellular space, and finally into the cerebrospinal fluid(CSF) and blood, is a direct measure of neuronal injury. The aim of this study is to determine the diagnostic role of NFL levels in CSF in newly diagnosed MS, pre-diagnosed MS and other diseases that are in the differential diagnosis of MS. **Methods:** There are three groups in this study: Newly diagnosed MS(Group A)(n=23), pre-diagnosed MS(Group B)(n=20) and other diseases that are in the differential diagnosis of MS (Group C)(n=41). Group A consists of Relapsing-Remitting MS(n=19), Secondary Progressive MS(n=1), Primary Progressive MS(n=1), Radiologically Isolated Syndrome(n=1), Clinically Isolated Syndrome (n=1) subgroups. The diagnosis of MS was confirmed by a neurologist using the revised 2017 McDonald criteria. Subgroups such as newly diagnosed malignant and benign diseases of CNS, normal pressure hydrocephalus and benign intracranial hypertension constitute the other diseases group that are in the differential diagnosis of MS(Group C). After the CSF samples were drawn by the neurologist, they were processed according to the consensus protocol previously determined by Teunissen et al.The ELISA kit from UMAN Diagnostics, a previously validated CSF kit, was used to determine CSF NFL levels. Serum samples of the patients participating in this study were also collected and planned to be analyzed later. Serum samples will be analyzed with Single Molecule Array(SIMOA) technology. Clinical and MRI information of the patients will be included in the statistical analysis at the end of the prospective study. SPSS 25 was used in all statistical analyses. **Results:** When the three groups were evaluated in terms of NFL levels(pg/mL), there was no statistically significant difference(p=0,052). The mean NFL value of group C(mean=3597,2 ; median=1169,6) was higher than the other two groups (Group A(mean=1102,1; median=848,1), Group B (mean=1200,9 ; median=706,8)). When Group A + B (mean=1146,8 ; median=796,4) and Group C(mean=3597,2 ; median=1169,6) were compared in terms of NFL levels, a statistically significant difference was found (p=0,001). The mean NFL values of CNS malignant diseases (mean=13615,4; median=13562,0), one of the subgroups that make up Group C, were found to be higher than group A and group B. There was no significant difference between the genders in terms of NFL levels (p>0,05). **Conclusion:** As a result of this study, it was revealed that NFL levels can be used not only in MS and related neuroimmunological diseases but also in CNS malignancies. Micrometastases that cannot be detected by imaging methods will perhaps make the NFL an important biomarker in the follow-up of these patients.

B-031

Performance of Hepatitis and Retrovirus Infectious Disease Assays on the Alinity i Analyzer

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Background: The performance of multiple infectious disease assays was evaluated in a multi-center study by performing reproducibility and method comparison between the Alinity i analyzer and the ARCHITECT i2000SR system. The objective was to demonstrate equivalent performance between the two instrument systems on 7 assays. The study was performed based on *Assay Migration Studies for In Vitro Diagnostic Devices Guidance for Industry and FDA Staff*, issued April 25, 2013. **Methods:** Reproducibility was assessed using multiple panel/control members for 5 days based on guidance from Clinical and Laboratory Standards Institute EP05-A2 and EP15-A2. Method comparison was assessed using specimens to target results at the low negative, high negative, low positive and moderate, and upper range of the assays. Negative and positive percent agreement were calculated. Studies for all assays utilized 1 lot of reagents, calibrators and controls. Testing was completed using 1 Alinity i analyzer at each of 3 clinical sites and 1 ARCHITECT i2000SR from one of the clinical sites. **Results:**

Assay Name & cutoff	Alinity iWithin-LaboratoryPrecision3 sites, 1 Lot	ARCHITECT i 2000SRWithin-LaboratoryPrecision1 site, 1 lot	Negative Percent Agreement (NPA)Positive Percent Agreement (PPA)
Hepatitis			
Anti-HCVNR: 0.00-0.79 S/COGZ: 0.80-0.99 S/COR: >/= 1.00 S/CO	Positive: 3.2–3.9 %CVNegative: 0.008–0.026 SD	Positive: 3.5–3.9 %CVNegative: 0.005–0.028 SD	NPA: 95.38% PPA: 98.33%
Anti-HBsNR: <8.00 IU/mLGZ: 8.00 - <12.00 IU/mL R: >/= 12.00 mIU/mL	Positive: 2.6–3.2 %CVNegative: 0.104–0.317 SD	Positive: 3.5–4.2 %CVNegative: 0.055-0.282 SD	NPA: 99.66% PPA: 99.54%
HBsAg Qualitative IINR: <1.00 S/COR: >/= 1.00 S/CO	Positive: 3.0 – 5.0 %CVNegative: 0.052–0.053 SD	Positive: 2.4–6.9 %CVNegative: 0.043-0.080 SD	NPA: 99.39% PPA: 100.00%
HBsAg Qualitative II Confirmatory	Not performed	Not performed	PPA: 100.00%
Anti-HBcNR: <0.80 S/COGZ: 0.80 - <1.21 S/COR: >/=1.21 S/CO	Positive: 2.3–2.5 %CVNegative: 0.010–0.019 SD	Positive: 1.5–2.1 %CVNegative: 0.007-0.018 SD	NPA: 97.12% PPA: 98.37%
Anti-HBc IgMNR: <0.80 S/COGZ: 0.80 - <1.21 S/COR: >/=1.21 S/CO	Positive: 3.0 –3.5%CVNegative: 0.004–0.026 SD	Positive: 3.8–5.1 %CVNegative: 0.005-0.038 SD	NPA: 100.00%PPA: 99.29%
Retrovirus			
HIV Ag/Ab ComboNR: <1.00 S/COR: >/=1.00 S/CO	Positive: 2.7–3.9 %CVNegative: 0.012–0.026 SD	Positive: 4.3–5.8%CVNegative: 0.012–0.041 SD	NPA: 100.00% for HIV Negatives, HIV Ag Viral Isolates PPA: 100.00% for HIV-1 Ab, HIV-2 Ab, HIV Ag Viral Isolates

NR: Nonreactive; GZ: Grayzone; R: Reactive

Conclusion: Equivalency was demonstrated between the Alinity i analyzer and the ARCHITECT i 2000SR System for these infectious disease assays.The study was funded by Abbott Laboratories.

B-032

Performance of Selected Hepatitis/ HIV Immunoassays on the Alinity i Analyzer, Abbott's Next Generation Immunochemistry System

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Background: Abbott offers a broad Alinity i infectious disease menu of assays that deliver reliable and accurate results for diagnosis and monitoring of hepatitis- and human immunodeficiency (HI) virus infections. Detection of respective diagnostic markers for HIV (HIV1/HIV2, core protein p24), hepatitis B (HBsAg, anti-HBc IgG/IgM, anti-HBs) and hepatitis C (anti-HCV) is crucial to identify individuals that can transmit the infection and to differentiate between acute, chronic or resolved infections. The objective of the presented studies is to evaluate the key performance characteristics of the abovementioned infectious disease assays developed for the new Alinity i system. Key assay characteristics include within-laboratory precision, analytical sensitivity, limit of blank (LoB)/ detection (LoD)/ quantitation (LoQ), seroconversion sensitivity, clinical sensitivity and clinical specificity. **Methods:** Within-

laboratory precisions were conducted based on guidance from CLSI EP05-A2 and CLSI EP05-A3. Analytical sensitivity was evaluated using serial dilutions of external standards and limits of blank/detection/quantitation were determined based on guidance from CLSI EP17-A2. Seroconversion panels obtained from commercial vendors were tested on the Alinity i analyzer and evaluated against the respective ARCHITECT comparator assay. Sensitivity* studies were conducted based on guidance from CLSI EP12-A2. For specificity* testing donor specimens were tested on the Alinity i analyzer and compared to the respective ARCHITECT assay. **Results:** Obtained results for within-laboratory precision, analytical sensitivity, lower limits of measurement, seroconversion and clinical specificity/sensitivity are summarized in the table below. **Conclusion:** The presented Alinity i assays demonstrated satisfactory within-laboratory precision and lower limit of measurement results. Seroconversion sensitivity, clinical specificity and sensitivity studies showed equivalent performances towards the respective ARCHITECT comparator assays.

Assay	Within-Laboratory Precision (Positive Samples %CV / Negative Samples SD)	Analytical Sensitivity / Limit of Detection	Seroconversion Sensitivity (Alinity i vs. ARCHITECT)	% Specificity* (Alinity i / ARCHITECT) IR (% of Total)	% Sensitivity* (Alinity i / ARCHITECT)
HIV Ag/Ab Combo	2.9 - 4.7 %CV / 0.011 - 0.035 SD	20.41 - 20.81 pg/mL	Equivalence across 37 seroconversion panels	99.93% / 99.91%; IR: 0.13%	100.00% / 100.00%
HBsAg Qualitative II / HBsAg Qualitative II Confirmatory	2.8 - 4.2 %CV / 0.048 - 0.053 SD	0.020 - 0.021 IU/mL (LoD: 0.002 - 0.012 IU/mL)	Equivalence across 32 seroconversion panels	99.96% / 99.96%; IR: 0.06%	100.00% / 99.80%
Anti-HBc	1.8 - 2.0 %CV / 0.009 - 0.018 SD	0.61 IU/mL	Equivalence across 8 seroconversion panels	N/A	N/A
Anti-HBc IgM	3.5 - 4.1 %CV / 0.005 - 0.031 SD	N/A	Equivalence across 9 seroconversion panels	100.00% / 100.00%; IR: 0.00%	100.00% / 100.00%
Anti-HBs	2.1 - 3.2 %CV / 0.096 - 0.293 SD	LoB: 0.33 mIU/mL; LoD: 0.51 mIU/mL; LoQ: 3.31 mIU/mL	N/A	N/A	N/A
Anti-HCV	3.2 %CV / 0.005 - 0.025 SD	N/A	Equivalence across 22 seroconversion panels	N/A	N/A

IR: Initial Reactive; N/A: Not applicable. *Tested on Alinity i out of US assays sharing the same formulation as the respective US assays.

B-033

Performance of Alinity i AFP Assay

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Background: The performance of the Alinity i AFP assay was evaluated in a multicenter study by performing reproducibility and method comparison between the Alinity i analyzer and the ARCHITECT i2000SR system. The objective was to demonstrate equivalent performance between the two instrument systems. The study was performed based on *Assay Migration Studies for In Vitro Diagnostic Devices Guidance for Industry and FDA Staff*, issued April 25, 2013. **Methods:** Reproducibility was assessed using multiple panels/control members based on guidance from Clinical and Laboratory Standards Institute EP05-A2 and EP-15-A2. Three controls and 5 serum panels were assayed in replicates of 4 at 2 separate times per day for 5 days. Method comparison was performed based on guidance from Clinical and Laboratory Standards Institute EP09-A3 using weighted Deming regression method. The study utilized 1 lot of reagents, calibrators and controls. Testing was completed using 1 Alinity i analyzer at each of 3 clinical sites and 1 ARCHITECT i2000SR from one of the clinical sites. **Results:**

Reproducibility - Alinity i Analyzer and ARCHITECT i2000SR System

	Alinity i AFP3 sites, 1 Lot						ARCHITECT i2000SR AFP1 site, 1 lot					
Sample	N	Meanng/mL	Within-Run		Within-Lab (Total)		N	Meanng/mL	Within-Run		Within-Lab(Total)	
			SD	% CV	SD	% CV			SD	% CV	SD	% CV
Panel 1	120	2.91	0.089	3.0	0.103	3.6	40	2.72	0.057	2.1	0.069	2.5
Panel 2	120	11.41	0.232	2.0	0.276	2.4	40	10.98	0.245	2.2	0.269	2.4
Panel 3	120	611.27	12.719	2.1	13.591	2.2	40	616.64	17.525	2.8	18.642	3.0
Panel 4	120	1517.33	34.193	2.3	41.228	2.7	40	1511.82	38.176	2.5	38.900	2.6
Panel 5	120	1741.06	39.012	2.2	49.461	2.8	40	1724.63	61.650	3.6	61.917	3.6
Low Control	120	20.12	0.467	2.3	0.593	2.9	40	19.51	0.409	2.1	0.441	2.3
Medium Control	120	211.22	4.103	1.9	4.576	2.2	40	213.93	4.753	2.2	5.030	2.4
High Control	120	1019.96	20.062	2.0	23.753	2.3	40	1013.11	29.738	2.9	32.537	3.2

Comparison Between the Alinity i Analyzer and ARCHITECT i2000SR System

Matrix	N	Correlation Coefficient (r)	Intercept	Slope	Concentration Range (ng/mL)
Serum	213	0.999	0.41	0.99	3.99 - 1915.46
Amniotic Fluid	183	0.998	0.44	1.00	4.28 - 1948.40

Conclusion: Equivalency was demonstrated between the Alinity i analyzer and the ARCHITECT i 2000SR System for the AFP assay.The study was funded by Abbott Laboratories.

B-035

Development of a New Architect Automated IL-6 Immunoassay

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Background: Interleukin-6 (IL-6) is a cytokine with diverse biological functions. IL-6 production is induced rapidly in acute inflammatory events and thus is suitable as a biomarker in serum and plasma for monitoring patients with disorders associated with acute inflammation and is beneficial in guiding patient management. **Methods:** Fifteen in-house and six external antibodies were screened on the ARCHITECT, an automated immunoassay instrument, with a total of 441 antibody pairs. The best antibody pair was selected and used to develop a prototype immunoassay that was assessed for sensitivity, specificity and spike recovery. Calibrator and panel preparations using an internal recombinant IL-6 were compared to serum panels prepared with the IL-6 International Standard 89/548.

Results: Capture and detection monoclonal antibodies were selected for the ARCHITECT IL-6 assay from our internal collection. The prototype assay showed high sensitivity with an estimated detection limit of 0.317pg/mL compared to 1.5pg/mL reported by Roche Cobas. Spike recovery of ARCHITECT IL-6 immunoassay was 90 - 110% in serum and plasma. Our internal recombinant human IL-6 calibrator showed excellent stability for 63 days at 2-8°C compared to only 5 hours for the Roche Cobas calibrator. ARCHITECT IL-6 immunoassay was specific for IL-6 and exhibited no cross reactivity to a variety of related cytokines and interleukins even at 50ng/mL.

Conclusion: These studies indicate that the prototype ARCHITECT IL-6 automated immunoassay is a reliable and robust method for the quantitative determination of IL-6 in human serum and plasma.

B-038

Analytical Performance of BÜHLMANN fPELA® Turbo, a Fecal Pancreatic Elastase PETIA Assay

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Background: Fecal pancreatic elastase is a proteolytic enzyme playing an important role in the digestive process of humans. High stability of the enzyme during intestinal transit makes pancreatic elastase a useful marker for the assessment of pancreatic function in patients suffering from conditions such as chronic pancreatitis or cystic fibrosis. Analytical performance of a new particle enhanced turbidimetric immunoassay (PETIA) was evaluated on the clinical chemistry analyzer Roche cobas c501.

Methods: New turbidimetric fecal elastase assay applies particles coated with anti-human pancreatic elastase antibodies. Pancreatic elastase levels were measured in extracts of human stool samples collected with the BÜHLMANN CALEX® Cap device. Analytical sensitivity study was performed according to CLSI guideline EP17-A2. For linearity serial dilutions were analyzed according CLSI EP6-A. Intra- and inter-assay precision was evaluated in agreement with CLSI EP5-A3. Reproducibility testing was performed on Roche c501, Beckman Coulter AU480, Mindray BS380 instruments. For method comparison >100 fecal patient samples were analyzed on Roche cobas c501(new PETIA) and Dynex DS2 (competitor ELISA) according CLSI guideline EP09-A3. Instrument variation was conducted with 49 samples on analyzer Roche cobas c501 and Mindray BS380.

Results: Analytical sensitivity was determined as 0.80 µg/g LoB, 2.33 µg/g LoD and 5.73 µg/g LoQ. Linear range is up to 5000 µg/g. Samples with theoretical concentrations of up to 14'444 µg/g can be measured without limiting the measuring range. Including a prozone check extends the testing range up to 20'783 µg/g. Within-lab precision was <2.2% CV, reproducibility, including three lot/instrument combinations ranged between 1.9 and 6.2% CV. Method comparison of the new PETIA with competitor ELISA assay revealed a slope of 0.9031 (Passing-Bablok), a bias at cutoff (200 µg/g) of 2.6% as well as a diagnostic agreement of >80%. Relative bias (instrument variation, Bland-Altman) of the two analyzer was 2.82%.

Conclusion: The new BÜHLMANN fPELA® turbo, turbidimetric assay represents an accurate, fast and precise method to determine pancreatic elastase levels in fecal extracts. BÜHLMANN fPELA® turbo is an attractive alternative to ELISA, it will improve the laboratory capability through reduced turn-around times, improved workflow management for random access and high throughput sample processing.

B-040

New Biotin Interference Free VITROS® MicroWell Technology in Next Gen Immunoassays on Ortho VITROS 5600/XT 7600 Integrated Systems and VITROS 3600 and VITROS ECi/ECiQ Immunodiagnostic Systems

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Background: The use of over the counter (OTC) biotin supplements has become increasingly prevalent in the last decade. Biotin doses of 2-20 mg/day can cause significant interference with heterogeneous immunoassays that utilize streptavidin-coated wells or particles. The presence of biotin in patient samples will compete with the biotin conjugate for streptavidin binding sites, resulting in suppressed signal and inaccurate results. Alternative surface immobilization methods are possible (i.e. adsorption or covalent attachment), but can often result in reduced antibody reactivity, which in turn compromises analytical performance. The ideal approach would preserve the benefits of indirect antibody attachment to the surface, while avoiding biotin interference. The new biotin interference free VITROS MicroWell technology eliminates biotin risk to results, as demonstrated by the new VITROS TSH3*, VITROS NT-proBNP II*, VITROS hsTnI* and VITROS PCT* assays. **In development.* **Methods:** VITROS MicroWell technology utilizes a coated-well format to accomplish the bound-free separation that is required by heterogeneous immunoassays. Detection is achieved using horseradish peroxidase (HRP) as the enzyme label. An antibody-HRP conjugate generates the assay signal when it is added into the coated wells. After completion of the binding reaction and a subsequent wash step to remove unbound enzyme label, a chemiluminescent substrate is added to the well to generate light. Legacy VITROS MicroWell assays have employed two different types of coated wells: the traditional ELISA method of direct adsorption to immobilize either antibody or antigen on the well surface (e.g. VITROS HCV), or a generic streptavidin-coated well coupled with biotin-labeled antibody or antigen in the liquid reagents (e.g. VITROS TSH). The second approach is subject to biotin interference, as biotin in the sample will compete with the biotin conjugate in the reagent. Two different coated-well formats have been developed for a biotin interference free design. Both approaches pre-bind the antibody conjugate to the coated well surface. One approach utilizes a streptavidin-coated well which has been overcoated with the antibody-biotin conjugate (e.g. VITROS NT-proBNP II, VITROS PCT). The second approach is more novel in that the antibody is first covalently attached to streptavidin, then bound to biotinylated protein that has been adsorbed to the coated well surface (e.g. VITROS TSH3, VITROS hsTnI).

Results: Pre-binding the antibody conjugate to the coated well surface eliminates risk of biotin interference, while preserving the benefits of the biotin-streptavidin capture system. Irreversible binding of the antibody is virtually indistinguishable from covalent attachment methods, due to the high-affinity in the biotin-streptavidin system. Excellent analytical sensitivity can be achieved through the indirect attachment of the antibody to the well surface, as it preserves a high level of antibody reactivity. The success of this novel approach has been demonstrated in VITROS TSH3 and other recent VITROS Immunodiagnostics assays, and is being used for next gen assays in development.

Conclusion: In summary, the biotin interference free design preserves the advantages of the biotin-streptavidin architecture, such as excellent analytical sensitivity, by pre-binding the capture antibody or antigen onto the coated well surface using an irreversible biotin-streptavidin linkage.

B-042

Performance Evaluation of a C1q Assay for Use on the Binding Site Optilite Analyser

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Background: C1q is a subcomponent of C1 and is the initiating component of the classical complement pathway. The action of C1q is initiated by binding to CH2 domains of aggregated IgG and CH3 domains of IgM. The resulting conformational change of C1q activates C1r and subsequently C1s, beginning the classical cascade. Measurement of C1q in serum, lithium-heparin and EDTA plasma provides a useful marker for disorders such as immune complex disease, systemic lupus erythematosus (SLE), meningitis and hereditary deficiency of C1q. C1q deficiency is the most common deficiency of the three key C1 subcomponents (C1q, C1r and C1s) and any such pathology results in reduced activation of the complement cascade. Here we describe the evaluation of a C1q assay for use on the Binding Site's Optilite® analyser. The instrument is a random-access turbidimetric analyser, capable of on-board sample dilutions up to 1/10,000 and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes, which are automatically loaded and disposed of. The assay is compatible with serum, lithium-heparin and EDTA plasma matrices, has a standard measuring range of 30-300mg/L and an overall range of 11-450mg/L.

Method: Paired serum, lithium-heparin and EDTA plasma samples from 49 patients were run against the assay to determine if the different matrices contained equivalent concentrations of C1q. A linearity study was performed as per EP06-A using a patient serum sample. Interference testing was performed following EP07-A2 at a single serum concentration and a single plasma concentration, challenging with triglyceride (300mg/dL), haemoglobin (500mg/dL), Intralipid (1000mg/dL) and conjugated and unconjugated bilirubin (both 20mg/dL). A precision study was performed (EP05-A2), by testing 3 serum levels on a single analyser over 20 days, using a single kit lot. Comparison was made to the Binding Site Radial Immunodiffusion C1q assay, using clinical and normal samples (n=49, range 11 - 220mg/L).

Results: The matrix comparison results were assessed using Altman-Bland analyses and displayed an overall bias of no higher than -4.1% between each matrix, indicating equivalent C1q concentration. The assay demonstrated non-linearity of no higher than ±2% when comparing linear and polynomial fit over a range of 13mg/L - 350mg/L. All interference results were <±10% when compared to equivalent negative controls. The precision assessment displayed within-run, between-run, between day and total precision CV% of no higher than 2.9%. Good agreement was observed between the Optilite® and Radial Immunodiffusion assay when analysed via Passing-Bablok regression; y=1.03x + 4.48.

Conclusion: In conclusion, the C1q assay for use on the Optilite® provides a reliable and precise method for quantifying C1q in serum, lithium-heparin and EDTA plasma samples and correlates well with existing methods.

B-043

Robust Molecularly Imprinted Polymers for Diagnostics

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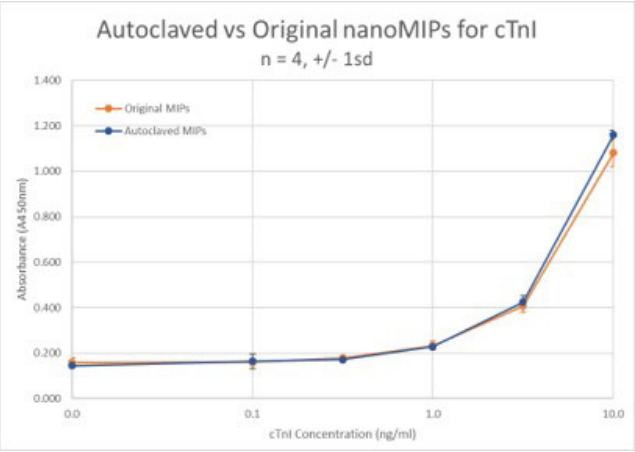
Background: MIP Diagnostics Limited produces nanoMIPs (nano-sized molecularly imprinted polymers) that are suitable for use in clinical diagnostic systems as a synthetic alternative to antibodies. They have unique properties compared to traditional binding molecules, not least the ability to be autoclaved and still retain functionality.

Methods: nanoMIPs specific to a cardiac marker - Troponin I - were synthesized using proprietary methodology, whereby a peptide specific to a region of the whole molecule was immobilised on a solid phase, monomers and cross-linker were added, controlled polymerisation was initiated and, ultimately, nanoMIPs with high affinity for Troponin I were eluted. Samples of the nanoMIPs were taken through one full autoclave cycle (121C, 210kPa, 15 minutes), before immobilising onto carboxy microtitre plates via EDC/NHS coupling along with an original (control) sample of the nanoMIPs that had not been autoclaved. An ELISA (enzyme-linked immunosorbent assay) was then performed using ITC Troponin Complex and a pairing antibody-HRP conjugate, with TMB substrate for colorimetric detection at 450nm.

Results: Autoclaved nanoMIPs performed equally well to original nanoMIPs for Troponin I in ELISA format.



Conclusion: MIP Diagnostics proprietary nanoMIPs specific for Troponin I have been shown to survive an autoclave cycle with full functionality demonstrated in a subsequent ELISA assay. The data generated would infer a very long shelf-life under ambient conditions. nanoMIPs therefore provide assay developers with a new, significantly more robust, option for producing assays: this could lead to a new generation of point of care and decentralised testing with extended shelf life and no need for controlled shipping and storage.

B-047

Performance of a Multiplex Method for Treponemal and Non-Treponemal Tests in Syphilis Diagnosis

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Background: Syphilis is a sexually transmitted disease caused by Treponema pallidum. It can be transmitted to the fetus during pregnancy and also plays a role in HIV spread. The clinical manifestations are variable and might evolve through different stages. The number of cases in Brazil has increased in recent years. Laboratory diagnosis is crucial for confirmation and monitoring of the disease. For this reason, a Multiplex assay performance was compared to the tests currently used at our laboratory, as an option of a fully automated diagnostic method in high-demand laboratories.

Methods: Serum samples with known treponemal (Chemiluminescence and IgG FTA-Abs) and non-treponemal flocculation (RPR) test results were analyzed by a Multiplex assay. The Multiplex assay is a dual treponemal/non-treponemal immunoassay that offers a one-step testing for syphilis, for the qualitative detection of total (IgG/IgM) antibodies and the titer determination of non-treponemal reagin antibodies.

Results: In the first validation step, the treponemal Multiplex assay performance was evaluated in 67 samples. In the second step, the non-treponemal Multiplex assay performance was evaluated in 69 samples. In the first step, the positive results obtained were: chemiluminescence in 56, Multiplex in 51, and IgG FTA-Abs in 45 samples. The negative results were: chemiluminescence in 11, Multiplex in 15, and IgG FTA-Abs in 22 samples. An indeterminate result was obtained in 1 sample by the Multiplex assay. The agreement rate observed between the tests was: chemiluminescence and Multiplex (62/67) = 92.5%; chemiluminescence and IgG FTA-Abs (56/67) = 83.6%; Multiplex and IgG FTA-Abs (60/67) = 89.5%. Five chemiluminescence positive samples with discrepant Multiplex results (4 negative and 1 indeterminate), showed an index value between 1.00 and 1.99. A sample with an index value of 2.00 in chemiluminescence, showed a Multiplex low positive result. The results in the second step were: positive flocculation test (RPR) in 40 and negative in 29 samples; positive Multiplex assay in 33 and negative in 36 samples. The agreement rate observed between the tests was 90.0% (62/69). Among the discrepant results (7/69), all Multiplex assay negative, the flocculation test (RPR) titers were 1/1 in 4, 1/2 in 2 and 1/4 in 1 samples. These 7 discrepant samples were positive in the treponemal tests. Among the positive results in both non-treponemal assays (33 samples), the same titer was obtained in 22, a one-titer difference in 10, and a two-titer difference in 1 sample. The titer determination agreement rate was 97.0% (32/33) considering a difference of up to one titer as acceptable.

Conclusion: The Multiplex assay performance in this study was adequate with an agreement rate greater than 90% between chemiluminescence and Multiplex assay. The IgG FTA-Abs, a subjective test, showed an agreement rate below 90%. An additional study is required to define a gray zone in chemiluminescence due to the discrepancies observed between the treponemal tests (index value between 1.00 and 1.99 are considered positive by the manufacturer). Discrepant results obtained between non-treponemal tests suggest that using manual methods in a high-demand laboratory might be critical.

B-049

Prevalence of ANA HEp-2 Patterns in Anti-dsDNA Positive Samples in Sao Paulo - Brazil

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Background: Autoantibodies against cellular antigens are considered the hallmark of autoimmune rheumatic diseases and the indirect immunofluorescence assay in HEp-2 cells (HEp-2 ANA) is the standard method for their detection. The patterns observed in microscopy suggest the type of antibody prevalent in serum and in general, patients diagnosed with systemic lupus erythematosus (SLE) have positive ANA test. Another important marker associated with SLE is the anti-double stranded DNA antibody (anti-dsDNA). The most common pattern observed in the HEp-2 ANA, associated with anti-dsDNA positivity is the Nuclear Homogeneous, however other patterns can be found. The purpose of the study was to evaluate the prevalence of HEp-2 ANA patterns in anti-dsDNA positive serum in the State of Sao Paulo-Brazil.

Methods: the study was carried out by surveying the database of a large laboratory located in Sao Paulo-Brazil, from January to June 2019. We select 237 HEp-2 ANA and anti-dsDNA positive samples in patients with no defined clinic. HEp-2 ANA and anti-dsDNA tests were performed by Indirect Immunofluorescence (IIF) method, following the manufacturer's recommendations, with initial dilution of 1:80 (HEP-2 ANA) and 1:10 (anti-dsDNA - *Crithidia luciliae*). Positive samples were performed in serial dilutions to obtain the final titer.

Results: in 237 samples analyzed, 215 (90.71%) are from women and 22 (9.29%) from men, with a mean age of 37 years old. The most frequent pattern was the nuclear homogeneous in 126 (53.16%) samples, followed by mixed patterns associated with the nuclear homogeneous in 52 (21.94%) samples. The nuclear large/coarse speckled pattern was observed in 26 (10.97%), and the nuclear fine speckled pattern in 22 (9.28%) samples. The other patterns were: 5 (2.10%) nuclear quasi-homogeneous pattern, 4 (1.68%) cytoplasmic dense fine speckled pattern and 2 (0.84%) nuclear pleomorphic speckled (PCNA-like).

Conclusion: the ANA pattern most frequently associated with positive anti-dsDNA was the nuclear homogeneous, however other patterns such as the nuclear fine speckled and nuclear large/coarse speckled were observed. This association also occurred in a small number of samples with other ANA patterns, such as nuclear quasi-homogeneous, cytoplasmic dense fine speckled and nuclear pleomorphic speckled (PCNA-like).

B-050

Development and Evaluation of Novel MAS™ Infectious Controls for Serology Diagnostic Tests

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Background: Human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis remain major infections around the world. Serological assays have revolutionized infectious disease testing and therapeutic monitoring for determination of the prevalence of these infections, led to better diagnoses and treatment plans for patients. The Thermo Scientific™ MAS™ Omni™ Infectious Controls containing single or multiple analytes in human plasma based matrix are quality controls mimicking patient samples to monitor serological assay performance; therefore, providing additional confidence in routine test results, test methods troubleshooting, and system errors identification. We report here the development and performance evaluation of the MAS Omni Infectious Controls.

Methods: Five MAS Omni Infectious Controls were developed and characterized: BSI (Bloodstream Infectious) Positive Control Panel, HIV p24 Positive Control, Syphilis Positive Control, anti-HBs Positive Control, and Negative Control. BSI Positive Control Panel is a multi-constituent quality control containing anti-HIV-1/2, anti-HBc, anti-HCV, and anti-HTLV I/II antibodies, as well as Hepatitis B surface antigen. The three single analyte positive controls were developed for the assessment of assay performance detecting anti-Treponema pallidum, anti-HBs, and HIV p24 antigen, respectively. The Negative Control is designed to be non-reactive to anti-HIV 1/2, anti-HTLV I /II, HBsAg, anti-HCV, anti-HBs, and anti-T. pallidum. The Negative Control diluent matrix is comprised of defibrinated, delipidized normal human plasma with preservatives. Performance evaluation are performed on multiple commonly used and most up-to-date clinical diagnostic platforms including Abbott™ Alinity™ and SIEMENS™ Atellica™ analyzers. Shelf life of the products are determined by accelerated and real-time stability monitoring. In-use stability and open vial stability monitoring are also performed to meet the customer needs.

Results: Five MAS Omni Infectious Controls were developed and evaluated with IVD assays. All five products demonstrated compatible performance across different clinical diagnostic platforms. Stability studies demonstrated a minimum of 24 or 18 months shelf life, 30 days of open-vial stability, and 8 hours of in-use stability is guaranteed when stored at 2-8 °C, respectively.

Conclusion: Five MAS Omni Infectious Controls that mimic patient samples have been developed as external controls for the assessment of assay performance in clinical laboratories for hepatitis B and C, HIV, and syphilis diagnostics. These reliable and stable quality control materials may support and enhance the efficiency of serology test method development, validation and verification, and routine assessment of clinical diagnostic assays.

B-052

Interpreter Training Improves Sensitivity of Immunotyping for the Detection of Monoclonal Immunoglobulins

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Background: Immunotyping (IT) uses antisera against IgG, IgA, IgM, kappa and lambda to shift the migration of immunoglobulins on capillary electrophoresis. Disappearance of the abnormality in the antiserum-treated pattern compared to the reference trace indicates the presence of a monoclonal protein (M-protein) and allows identification of its isotype. IT offers several advantages over immunofixation (IF) such as higher throughput, automation, and digital results. However, previous studies have shown the sensitivity of IT to be inferior to IF. Since pathologists and chemists are often more familiar with interpreting IF compared to IT, it has been hypothesized that lack of training and experience may explain the lower analytical sensitivity observed in previous studies. The purpose of this study was to evaluate if training improves the analytical sensitivity of IT.

Methods: Patient serum samples (N=360) were obtained from the clinical laboratory at Memorial Sloan Kettering Cancer Center (MSKCC). The study included any sample that had serum protein electrophoresis (SPEP), IF and free light chain studies ordered for clinical purposes. Samples were stored at 4°C for up to 5 days prior to IT analysis on a CAPILLARYS 3 Tera instrument (Sebia, Inc.). To evaluate the impact of training on IT sensitivity, 3 MSKCC attendings independently interpreted the 360 IT results. MSKCC reviewers were self-taught and had minimal IT-reading experience (<5 IT/week for clinical purposes). Samples 1-120 were used to establish baseline performance. MSKCC reviewers were then trained by the Result Interpretation Escalation Team (RIEscalation Team) at Sebia. Samples 121-240 were used for practice, and samples 241-360 were used to test the hypothesis. For comparison, the 360 IT results were also independently interpreted by 3 experienced reviewers from Sebia's RIEscalation Team. Finally, IF results for these patients were independently interpreted by the 3 MSKCC attendings. This was a blinded study in which the reviewers did not have IT results, IF results or patient history when interpreting each test. The sensitivity of all interpretations (i.e. MSKCC IT, expert IT, MSKCC IF) were compared to the "gold standard", which was determined from review of patient history, clinical SPEP, IF and free light chain results.

Results: Prior to training, the average IT sensitivity for the 3 MSKCC attendings was 65% (range: 61-68%) compared to the gold standard. After formal training and practice with 120 samples, the sensitivity of the MSKCC reviewers was 83% (79-87%), an improvement of 18%. For reference, the average sensitivity of expert IT review (3 reviewers from Sebia's RIEscalation Team) was 92% (91-95%) and the average sensitivity of MSKCC IF review was 92% (90-93%).

Conclusion: Training and practice with 120 samples improves the analytical sensitivity of IT for the detection of monoclonal proteins. Additional experience with IT interpretations is likely needed for newly-trained reviewers to match the sensitivity of "expert" IT reviewers or with IF. Sensitivity may also be improved if results are reviewed with others to reach a consensus call.

B-053

Screening for Anti-Nuclear Antibody (ANA) - Utility of Enzyme Immunoassay Containing a Limited Number of Autoantigen Targets

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Background: Screening for anti-nuclear antibody (ANA) is frequently requested when providers suspect the presence of a connective tissue disorder (CTD). The most common methods are indirect immunofluorescence (IIF) and immunoassay, either enzyme immunoassay (EIA) or multiplexed immunoassay (MPIA). Some manufacturers have added additional autoantigens to immunoassay screens in an attempt to match the sensitivity of IIF. We sought to validate the performance of an EIA ANA screen with a limited number of targets. We compared it to an MPIA with a more extensive array, as well as IIF.

Methods: The Phadia EIA assay sold in the U.S. (Thermo Phadia 250 EliA Symphony) contains only seven nuclear antigens: Sm, RNP, Ro, La, Scl-70, centromere B, and Jo-1. (The version available outside the U.S. contains seven additional antigens.) We compared the U.S. Phadia assay with the BioPlex MPIA screen (Bio-Rad), which contains all of the antigens present in the Phadia assay as well as several others including dsDNA, chromatin, and ribosomal P. 105 samples submitted for ANA screening were analyzed by both immunoassay screens, as well as by IIF (Grifols Helios). Discrepancies between the two immunoassay screens were investigated using chart review.

Results: Of the 105 samples, the agreement was 74/105 (70%). 32 were positive and 42 were negative by both immunoassay screens, and these results were confirmed by IIF. 6 were positive by MPIA for anti-dsDNA only and 3 were positive by MPIA for anti-chromatin only; negative EIA results for these were expected since neither antigen is part of the EIA screen. Considering only the seven antigens present in the EIA, 22 samples were discrepant, with positive results by MPIA and negative results by EIA. MPIA results producing these positive screens included anti-RNP (13), anti-Scl-70 (6), and anti-La (3). Levels ranged from 1.0-6.0 AI with a mean of 2.4 [cut-off: <1.0]. Among the 22 patients with unexplained discrepant results (14 female, 8 male, mean age 58), the most common chief complaint was arthralgia (n=12); other various complaints were non-specific. Two had a diagnosis of CTD (rheumatoid arthritis and discoid lupus erythematosus). Two other patients without a specific diagnosis displayed signs or symptoms suggestive of a CTD (photosensitivity, Raynaud's phenomenon). Nine of the patients with unexplained discrepant results were positive for ANA by IIF, including all four with either a diagnosis of CTD or signs suggestive of one. The other thirteen were negative for ANA by IIF, and lacked clinical evidence of CTD.

Conclusion: The overall agreement between the limited EIA screen and the more comprehensive one was 70%. Of the samples which were discrepant, 9 were attributed to either anti-dsDNA or anti-chromatin. 22 were discrepant because EIA was negative for antigens known to be present in the screen. However, over half of these were negative by IIF and most of the patients had no clinical evidence of CTD. We conclude that the U.S. version of the Phadia 250 assay is a useful ANA screen, but laboratories need to add the separate immunoassay for anti-dsDNA as well for optimal performance.

B-055

A Validation Study of a Novel poly-MALDI-TOF-MS Assay for the Detection of Monoclonal Proteins

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Background: The continuous development of novel therapies has improved response rates and clinical outcomes in patients with plasma cell dyscrasias. Recently a novel approach utilizing immunoglobulin isotype-specific nanobody enrichment coupled to matrix assisted laser desorption ionization time-of-flight mass spectrometry (NB-MALDI-TOF-MS) has been demonstrated to have favorable overall performance to standard electrophoretic methods for the identification of M-proteins. Here we examine the performance of an alternative mass spectrometry method using polyclonal sheep antibody-coated magnetic microparticles enrichment (poly-MALDI-TOF-MS) for the detection and isotyping of the M-protein. We describe the validation of these reagents using a Tecan Fluent 780 liquid handler and Shimadzu MALDI-TOF 8020 mass spectrometer for sample preparation and analysis, respectively, and compare the performance to immunofixation (IFE) and NB-MALDI-TOF MS.

Methods: De-identified, waste patient samples were obtained from the clinical laboratory at Memorial Sloan Kettering for this study. Immunoglobulins were purified from serum using modified sheep polyclonal antibodies (anti-IgG, -IgA, -IgM, -kappa and -lambda; The Binding Site Group Ltd., Birmingham, UK) which were covalently attached to blocked magnetic microparticles. The microparticles were incubated with patient sera, washed and treated with acetic acid (5% v/v) containing TCEP (20 mM) to elute free light chains in monomeric form. Mass spectra were acquired on a MALDI-TOF MS system (Shimadzu, UK). Patient samples with clinical IFE results (n=98) were used for comparison studies. We evaluated assay reproducibility using negative and positive control samples. The limit of detection was determined by serially diluting normal donor serum spiked with the therapeutic antibody, daratumumab. Stability of the analyte was tested at ambient temperature, 4°C, and -20°C for various time points. Interference due to hemolysis, lipemia, or icterus was evaluated on normal serum and patient samples. Finally, patients who were receiving daratumumab and had a history of IgG kappa monoclonal gammopathy were tested to determine assay specificity.

Results: Validation experiments for poly-MALDI-TOF-MS showed good between-run and within-run reproducibility for spot and sample preparation replicates. The assay was about 8 times more analytically sensitive than IFE in serial dilutions of the therapeutic antibody daratumumab, with a limit of detection of 0.01 g/dL. Hemolysis, lipid or bilirubin did not interfere with the detection or typing of M-proteins, and the sample analyte was stable for up to 20 days in samples stored at room temperature and 4°C; and for 7 weeks when stored frozen at -20°C. In patient samples (n=98), poly-MALDI-MS results were concordant to IFE for 83 samples. In 10 samples, MALDI found an additional M-protein that was not detected by IFE. In 5 samples, IFE found an additional M-protein that was not detected by MALDI. Results from poly-MALDI-MS matched those from NB-MALDI-TOF MS.

Conclusion: Poly-MALDI-TOF-MS demonstrates good analytical performance. Results are reproducible and compare well to IFE and to a nanobody-based MS method, making it a suitable high-throughput option for routine screening and monitoring of patients with plasma cell dyscrasias in the era of novel agents.

B-056

Evaluation of a Multiplex Syphilis Treponemal/Nontreponemal Assay and Comparison of Algorithms in a Low Prevalence Population

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Background: Laboratory diagnosis of syphilis is based on two types of serology tests (treponemal and nontreponemal) using either a traditional or reverse algorithm. This study evaluated the performance of an automated multiplex Syphilis Total & RPR assay and compared the two algorithms for syphilis screening. **Method:** Residual patient samples of syphilis tests were used for the assay validation. The Bioplex syphilis total antibody and RPR assay (Bio-Rad, CA) were compared to the Centaur Syphilis IgG (Siemens, NY) and VDRL assay (BD, CA) respectively. After the implementation of Bioplex assay, total antibody and RPR results of 6178 consecutive specimens submitted for routine syphilis testing at our medical center were assessed for the outcome of the reverse and traditional algorithm. All discordant samples were assessed by performing the Treponema pallidum particle agglutination (TP-PA; Fujirebio Diagnostics) and reviewing the chart. **Results:** Assay characteristics were summarized in the Table. Using the reverse algorithm, 2.5% (147/6178) were reactive for syphilis antibody and 30% (45 patients) of those were also reactive for RPR while 70% were discordant samples with non-reactive RPR. Further TP-PA test revealed 46% of the discordant samples were falsely positive and 54% (53 patients) were reactive. Based on clinical evidence, 55% (29/53) were classified as successfully treated syphilis and 45% (24/53) were classified as undiagnosed and untreated infections. The reverse algorithm showed 100% sensitivity and 99.2% specificity. Using the traditional algorithm, 5.8% (358/6178) were reactive by the initial RPR test. A total of 45 patients was screened positive for syphilis with a sensitivity of 65% and a specificity of 100%. Compare to the traditional algorithm, 24 additional syphilis patients were identified. **Conclusion:** The Bioplex assay provides an automated approach for syphilis screening with acceptable performance. The reverse algorithm demonstrated superior sensitivity to the traditional algorithm with good specificity.

Syphilis Total Antibody/RPR Assay performance Characteristics

Precision	**Syphilis total antibody**		**RPR Assay**	
Samples	Intra-assay	Inter-assay CV	Intra-assay CV	Inter-assay CV
Negative Pool	0%	9.1%	0%	7.1%
Positive pool	4.6%	3.9%	4.6%	10.1%
RPR titer precision (5 replicate per day for 5 days)			Titer 1:8 Titer 1:256	±0 titer ±1 titer
Method comparison	Syphilis antibody: Bioplex vs. Centaur (n=55)		Bioplex RPR vs. VDRL (n=143)	
Overall agreement	91%		94%	
Positive agreement	84% (27 of 32)		92% (22 of 24)	
Negative agreement	100% (20 of 20)		93% (111 of 119)	
RPR/VDRL titer agreement (n= 21)			Within ±1 titer Within ±2 titer Within ±3 titer	38% 52% 100%

B-058

Cross-Platform Evaluation and Performance Ofthyroid-Stimulating Hormone Receptor Antibody (TRAb) Immunoassays

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Background: Hyperthyroidism in Graves' disease is caused by autoantibodies that activate the thyroid-stimulating hormone receptor (TSHR), subsequently stimulating thyroid growth and thyroid hormone synthesis and secretion. TRAb detection in serum is used to distinguish Graves' disease from other causes of hyperthyroidism, assess risk of Graves' disease relapse following antithyroid treatment, and determine neonatal thyrotoxicosis risk of a pregnant female with active/past Graves' disease. We compared three commercially available immunoassays (BRAHMS TRAK Kryptor (Thermo Scientific), cobas e411, and cobas e602 Elecsys Anti-TSHR (Roche)) and evaluated the analytical performance of Kryptor and cobas e411 assays. **Methods:** A three-way method comparison (Kryptor (Thermo Scientific), cobas e411 (Roche), and cobas e602 (Roche)) was performed using 40 patient serum samples measured across 5 days. A subset of samples auto-diluted on Kryptor were confirmed by manual dilution. The imprecision of the Kryptor and cobas e411 assays were assessed by measuring two levels of quality control (QC) material twice per day over 20 days. To independently assess imprecision over time in an unbiased manner, QC materials from Roche were also evaluated on Kryptor and vice versa. The analytical measurement range was evaluated by using low and high pools of patient serum using six concentrations that span the claimed reportable limits, Kryptor (0.27-20 IU/L) and cobas e411(0.8-40 IU/L). **Results:** The Kryptor, cobas e411, and cobas e602 TRAb immunoassays correlated well (cobas e411 v. cobas e602 (r^2=0.997); cobas e602 v. Kryptor (r^2=0.906); cobas e411 v. Kryptor (r^2=0.912)). Furthermore, the absolute and percent bias between the methods was minimal (cobas e411 v. cobas e602 (0.40 IU/L (13.3%); Kryptor v. cobas e602 (1.82 IU/L (2.0%); Kryptor v. cobas e411 (1.41 IU/L (-7.5%)). When comparing auto-diluted values to those obtained after manual dilution

(n=6), the absolute and percent difference varied from -8.31-1.03 IU/L (average: -2.42 IU/L for manual dilution) and -12.0% to 5.0% (average: -4.67%), respectively. The imprecision for Kryptor was 13.6% at 2.46 IU/L (within-day: 10.3%) and 7.73% at 9.75 IU/L (within-day: 5.55%) using Kryptor QC material across 20 days and 5.91% at 3.49 IU/L (within-day: 5.26%) and 5.72% at 13.4 IU/L (within-day: 5.73%) using Roche QC material across 10 days. The imprecision for Roche was 9.00% at 4.41 IU/L (within-day: 8.90%) and 2.00% at 18.2 IU/L (within-day: 1.73%) using Roche QC material across 20 days and 4.81% at 3.59 IU/L (within-day: 4.67%) and 2.92% at 12.2 IU/L (within-day: 2.71%) using Kryptor QC material across 10 days. TRAb showed a nonlinear response on the cobas e411 and a linear response on the Kryptor within their respective claimed analytical measure range. **Conclusion:** Overall, our results demonstrate good comparability between three automated, commercially available TRAb immunoassays. Kryptor assay has limited calibration (5 days), reagent (5 days on board) and QC (24 hours at 2-8°C) stability. The Roche reagent has a 3-week on-board stability, while pretreatment reagent and QC are stable for 3 days on-board (longer with intermittent room temperature storage) and at 2-8°C, respectively. Hence, imprecision was within 15% on Kryptor and 10% on Roche using either supplier of QC material.

B-059

Evaluation of Fully Automated IFA ANA Analyzer in Real-life Laboratory Settings

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Background: Indirect immunofluorescence assay (IFA) on HEp-2 cells is the gold standard for antinuclear antibody (ANA) screening and titration. We aimed to evaluate the performance of a fully automated IIF ANA analyzer (HELIOS; Aesku, Germany) in our real-life laboratory settings.

Methods: A total of 3,276 consecutive serum samples were analyzed for ANA screening and titration using HELIOS from May to August 2019. The positive/negative results, patterns and end-point titers proposed by HELIOS were compared with those of visually interpreted by two experienced observers using images obtained from HELIOS. The working cut-off dilution was 1:80. The manufacturer's recommended cut-off value of light intensity for discriminating positive from negative results was 70. A receiver operating characteristic (ROC) with area under the curve (AUC) was conducted to determine the optimal light intensity cut-off value for HELIOS. The end-point titer on visual inspection was compared with the estimated end-point titer based on the light intensity using the screening mode of HELIOS software. Results within ± one titer were considered concordant. In addition, we compared the turnaround time and the number of wells used per test before and after the introduction of HELIOS.

Results: Based on the analysis of visual reading for positive/negative results, among a total of 3,276 samples, 748 were positive and 2,528 were negative. Using 70 as the light intensity cut-off, the overall sensitivity and specificity of HELIOS were 73.3% (548/748) and 99.4% (2,512/2,528), respectively, with an agreement of 93.4% (3,060/3,276; κ=0.795). However, using 57.9 as the optimal cut-off value based on the ROC curve analysis (AUC = 0.969, $P < 0.0001$), the overall sensitivity and specificity were 84.5% and 95.0%, respectively. For pattern recognition, HELIOS correctly identified 70.1% (298/425) of samples with single pattern as follows: 100% (2/2) with nuclear dots, 91.3% (21/23) with nucleolar, 77.5% (79/102) with homogeneous, 73.7% (151/205) with nuclear speckled, 69.7% (23/33) with centromere, 40.7% (22/54) with cytoplasmic, and 0% (0/1) with nuclear envelope. HELIOS correctly recognized at least one major pattern in 72.4% (89/123) of samples with mixed patterns. For titration ranging from 1:80 to 1:5120, there was an agreement of 75.6% (273/361) between estimated and visual end-point titers. Median turnaround time was 100.6 hours for manual versus 55.7 hours for HELIOS ($P < 0.0001$). The number of wells used per test was 4 for manual versus 1.5 for HELIOS.

Conclusion: HELIOS system, a fully automated IFA ANA analyzer, performs well for distinguishing between positive and negative results, in addition to its definite advantage in saving labor, time, and cost, although it has some limitations in pattern recognition and end-point titer estimation. We hope that future technological advances will allow automated ANA processors to be fully integrated into the diagnosis process of the clinical laboratory.

B-060

Biomarkers for Detection of Mycobacterium Tuberculosis Infection in Supernatant of QuantiFERON Gold In-Tube and Gold Plus

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Background: We aimed to detect useful biomarkers for detecting Mycobacterium tuberculosis (Mtb)-infected cases, which include the latent TB infected and active TB patients.

Methods: A total of 38 background-corrected *Mtb*-specific antigen-stimulated and unstimulated cytokines, chemokines and growth factors were analyzed by Luminex assay in supernatants of QuantiFERON Gold In-Tube (QFT-IT) and Gold Plus (QFT-Plus) simultaneously. A total of 100 individuals consisting of 35 healthy controls, 30 latent TB infection subjects (LTBI) and 35 active pulmonary TB patients (ATB) were evaluated. Biomarkers were compared between multiple groups using Kruskal-Wallis tests. Mann-Whitney U tests were used to compare biomarkers between two groups. Dunn's or Bonferroni tests were used for post hoc correction to account for multiple comparisons, respectively. In order to define the diagnostic performance of selected biomarkers, receiver operator characteristic (ROC) curve analysis was performed.

Results: In QFT-IT, nine stimulated biomarkers were comparable between the *Mtb*-infected group (LTBI and ATB) and HC. In QFT-Plus which contains two antigen tubes (T1 and T2), 12 biomarkers of T1 and 14 biomarkers of T2 were significantly different between the two groups ($P < 0.0001$). The diagnostic potential of the biomarkers that were found to be capable of discriminating between the *Mtb*-infected and HC groups is shown in Table 1. IFN-γ showed the best performance characteristics in both QFT-IT and QFT-Plus. Generally, compared to QFT-IT, the biomarkers in QFT-Plus showed higher AUC values. Additional to IFN-γ, IL-2, TGF-α, IP-10, GM-CSF and IL-1RA showed good to excellent accuracy in discriminating between the *Mtb*-infected and HC.

Conclusion: Incorporating these selected biomarkers into future blood-based TB assays could result in substantial performance gains. Furthermore, combinations of these biomarkers could improve the ability to detect *Mtb*-infected individuals.

Table 1. Diagnostic performance of individual biomarkers in discriminating between *Mtb*-infected (LTBI and ATB) and HC.

QFT-IT				QFT-Plus T1				QFT-Plus T2			
Biomarker[a]	AUC	SN (%)	SP (%)	Biomarker[a]	AUC	SN (%)	SP (%)	Biomarker[a]	AUC	SN (%)	SP (%)
IFN-γ	0.88	85.0	94.3	IFN-γ	0.96	93.9	94.3	IFN-γ	0.95	89.2	91.4
IL-2	0.87	83.3	94.3	IL-2	0.94	89.2	94.3	IL-2	0.92	84.6	94.3
IP-10	0.83	76.7	88.6	TGF-α	0.90	81.5	91.4	TGF-α	0.90	78.5	100.0
IL-1RA	0.83	68.3	91.4	IP-10	0.90	95.4	71.4	IP-10	0.89	78.5	91.4
TGF-α	0.81	71.7	88.6	GM-CSF	0.89	81.5	88.6	GM-CSF	0.83	80.0	85.7
GM-CSF	0.81	66.7	100.0	MIP-1β	0.83	70.8	85.7	MCP-1	0.82	58.5	100.0
IL-8	0.77	83.3	71.4	IL-1RA	0.81	69.2	80.0	MIP-1β	0.82	69.2	94.3
MIP-1β	0.76	81.7	74.3	MCP-1	0.81	64.6	85.7	IL-1RA	0.80	66.2	85.7
IL-5	0.74	56.7	97.1	IL-5	0.77	63.1	88.6	MCP-3	0.79	72.3	77.1
				IL-8	0.74	72.3	77.1	IL-8	0.74	63.1	85.7
				MCP-3	0.73	78.5	68.6	IL-6	0.73	69.2	82.9
				IL-3	0.71	38.5	100.0	TNF-α	0.72	72.3	77.1
								IL-5	0.71	47.7	94.3
								IL-3	0.68	38.5	94.3

Mtb, Mycobacterium tuberculosis; LTBI, latent tuberculosis infection; ATB, active tuberculosis; HC, healthy controls; QFT-IT, QuantiFERON Gold In-Tube; QFT-Plus, QuantiFERON-Gold Plus; T1, Tube 1; T2, Tube 2; AUC, area under the curve; SN, sensitivity; SP, specificity.
[a] Biomarkers are shown in descending order of AUC values.

B-061

Anti-DFS70 Antibodies Detected as Part of a Particle Based Multi-Analyte Technology System Aids in the Referral of ANA Positive Individuals

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Background: The referral of patients with positive anti-nuclear antibody (ANA) tests to tertiary care specialists has been criticized as inappropriate use of medical resources thereby increasing the need to explore efficient serology testing alternatives. Multi-analyte technologies encompassing markers beyond the traditional extractable nuclear antigens (ENA), improve the diagnostic accuracy, turn-around-time, and potentially reduce the economic burden when implemented correctly. Anti-DFS70 antibodies without concomitant presence of disease-associated antibodies are not uncommon in ANA positive cases which further makes a case to compare the performance and utility of a novel particle based multi-analyte technology (PMAT) with a chemiluminescent immunoassay (CIA) for the detection of anti-DFS70 antibodies. **Methods:** Over a duration of three years, 15,357 patients were referred through a central triage (CT) system for review by tertiary care specialists. 643/15,357 (4.1%) were positive for HEp-2 ANA. 220/643 (34.2%) cases were further tested using PMAT and CIA (QUANTA Flash, Inova Diagnostics, San Diego, CA) systems for the presence of anti-DFS70 antibodies. In PMAT, antigens were coupled to encoded paramagnetic particles and run on the Aptiva system (Inova Diagnostics, research use only) using the CTD Essential reagent which detects autoantibodies to centromere B, dsDNA, Jo-1, ribosomal-P, RNP, Ro60, Ro52, Scl-70, Sm, SSB, and DFS70 simultaneously.

Results: Out of the 220 ANA positive individuals, 52 (23.6%), 16 (7.3%) and 152 (69.1%) were defined as ANA-associated rheumatic disease (AARD), unresolved, or non-AARD cases, respectively. Out of 220 ANA positive cases, 48 (21.8%) and 42 (19.1%) were anti-DFS70 positive on PMAT and CIA, respectively [total percent agreement 95.5% [95%CI: 91.8-97.5]; *kappa* of 0.86 [95% CI 95% CI 77.8-94.3] and spearman's rho of 0.642). All 21 (9.5%) monospecific DFS70 positive (by PMAT on tested panel) were positive on CIA for anti-DFS70. Moreover, 20/21 (95.2%) monospecific anti-DFS70 (by PMAT) cases were not associated with an AARD, OR 6.9 [95% CI 1.1-41.3].

Conclusion: This is the first study to evaluate the serological (on PMAT) and clinical features of patients referred through a CT system because of a positive ANA. Novel PMAT and established CIA systems showed a high level of agreement for anti-DFS70 positivity. All the monospecific anti-DFS70 positive cases by the PMAT system were positive on CIA and the majority were not associated with an AARD. These findings validate the utility of an expanded ENA panel that incorporates anti-DFS70 in the context of a large CT referred cohort.

B-062

Detection of Myositis Specific Autoantibodies on a Novel Fully Automated Random-Access Particle Based Multi-Analyte Test System

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Background: Myositis specific antibodies (MSA) represent important diagnostic tools and help stratify idiopathic inflammatory myositis (IIM) patients with particular clinical features, treatment responses, and disease outcomes. Standardization of MSA detection is of high importance because these antibodies also have the potential to be used in classification criteria. The objective of this study was to evaluate the clinical performance of a novel particle based multi-analyte technology (PMAT) for the detection of MSA

Methods: The study included 464 patient samples collected at Hospital Vall d'Hebron, Univeristy of Barcelona, most of whom had a diagnosis of IIM (n=264). Controls include samples from patients with myositis like conditions (ML, n=20), rheumatoid arthritis (RA, n=33), systemic lupus erythematosus (SLE, n=40), Sjögren`s syndrome (SjS, n=25), infectious diseases (ID, n=40) and healthy individuals (HI, n=42). All samples were tested using a novel fully automated particle-based multi-analyte technology (PMAT, Inova Diagnostics, research use only) that uses encoded paramagnetic particles with unique signatures and digital interpretation system. MSA panel consists of Jo-1, Mi-2b, TIF1y, PL-12, SAE, EJ, MDA5, HMGCR, PL-7, SRP and NXP2.

Results: The sensitivity/specificity of the individual MSA were: 19.7%/100% (Jo-1), 7.2%/100.0% (Mi-2), 3.0%/99.0% (NXP2), 3.8%/100.0% (SAE), 2.7%/100.0% (PL-7), 1.9%/99.5 (PL-12), 1.1%/100.0% (EJ), 15.5%/99.5% (TIF1y), 8.3%/98.5% (MDA5), 6.1%/99.0% (HMGCR) and 1.9%/98.5% (SRP). Of all IIM patients 136/262 tested positive for at least one of the MSA. In the individual control groups, 0/20 (0.0%) of ML, 2/33 (6.1%) of RA, 5/40 (12.5%) of SLE, 2/25 (8.0%) of SjS, 2/40 (5.0%) of ID and 1/42 (2.4%) of HI were positive for at least one MSA. Most of the control samples that tested positive had levels close to the cut-off (except one SRP and one PL-12). Only 6/262 (2.2%) IIM patients were positive for more than one antibody (MDA5/HMGCR, EJ/PL-7, 2 x MDA5/TIF1y, EJ/SAE, SAE/TIF1y). The overall diagnostic performance was: Sensitivity 68.2% (95% Confidence interval 62.3-73.5%), specificity 94.0% 95% CI 89.8-96.5) and odds ratio 33.8.

Conclusion: The novel PMAT used to detect a spectrum of MSA in IIM on a fully automated system. Sensitivity and specificity were in the expected range and in line with the known clinical associations of MSAs.

B-063

Clinical Evaluation and Method Comparison of Novel Assays for the Detection of Antibodies Associated with Celiac Disease

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Background: Antibodies to tissue transglutaminase (tTG) and deamidated gliadin peptide (DGP) are important factors in diagnosis of celiac disease (CD). Increased anti-tTG IgA titers can be especially important as suggested by the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), where a titer 10 times the upper limit of normal may consider foregoing invasive intestinal biopsy in diagnosis of CD. Recently, novel assays have been developed which allow for the detection of antibodies to tTG and DGP. This study aimed to compare the performance of novel tTG and DGP assays with reference methods using clinically characterized samples. **Methods:** A total of 461 samples were included in the study, consisting of 161 samples from CD patients, and 290 samples from patients with various other diseases including other gastroenterological conditions (including rheumatoid arthritis,

ulcerative colitis, Sjogren's syndrome, system sclerosis, autoimmune gastritis, systemic lupus erythematosus, and infectious disease samples). All samples were tested by a novel fully automated particle-based multi-analyte technology (PMAT, research use only, Inova Diagnostics, USA). Additionally, all samples were tested in parallel using chemiluminescent based assays currently on the market (QUANTA Flash, Inova Diagnostics, USA). Qualitative correlations were calculated, and clinical performance was assessed for each of the analytes. **Results:** The results derived from the clinical evaluation and the method comparisons are summarized in the tables below.

Table 1 - Clinical analysis and method comparison of the novel assays using all samples (n=461)

Analyte	tTG IgA	DGP IgA	tTG IgG	DGP IgG
Method comparison				
NPA % (95% CI)	99.7 (98.1-99.9)	99.4 (97.9-99.8)	95.1 (92.4-96.9)	93.9 (90.8-96.0)
PPA % (95% CI)	98.8 (95.6-99.7)	87.1 (79.8-92.0)	90.1 (82.3-94.7)	97.7 (93.5-99.2)
TPA % (95% CI)	99.3 (98.1-99.8)	96.3 (94.2-97.7)	94.1 (91.6-95.9)	95.0 (92.6-96.7)
Kappa (95% CI)	0.99 (0.97-1.00)	0.90 (0.85-0.95)	0.82 (0.76-0.89)	0.88 (0.84-0.93)
Aptiva clinical performance				
Sensitivity % (95% CI)	93.0 (88.1-95.9)	59.1 (51.6-66.2)	58.5 (51.0-65.6)	83.0 (76.7-87.9)
Specificity % (95% CI)	99.3 (97.5-99.8)	99.3 (97.5-99.8)	100.0 (98.7-100.0)	97.9 (95.6-99.0)
Likelihood ratio+ (95% CI)	134.83 (37.48-491.35)	85.64 (23.70-313.39)	+∞ (44.72-+∞)	40.14 (18.65-87.49)
Likelihood ratio - (95% CI)	0.07 (0.04-0.12)	0.41 (0.34-0.48)	0.42 (0.34-0.49)	0.17 (0.12-0.24)
Odds Ratio (95% CI)	1908.0 (456.3-7773.4)	207.77 (54.91-783.00)	+∞ (105.57 -+)	231.77 (95.66-558.73)
QUANTA Flash clinical performance				
Sensitivity % (95% CI)	93.0 (88.1-95.9)	64.9 (57.5-71.7)	50.3 (42.9-57.7)	74.3(67.2-80.2)
Specificity % (95% CI)	99.0 (97.0-99.6)	98.3 (96.0-99.3)	98.3 (96.0-99.3)	98.6 (96.5-99.5)
Likelihood ratio+ (95% CI)	89.88 (31.01-264.00)	37.65 (16.23-88.43)	29.10 (12.50-68.87)	53.85 (21.18-138.62)
Likelihood ratio - (95% CI)	0.07 (0.04-0.12)	0.36 (0.29-0.43)	0.51 (0.43-0.58)	0.26 (0.20-0.33)
Odds Ratio (95% CI)	1267.58 (367.65-4292.46)	105.45 (42.32-261.95)	57.67 (23.26-142.54)	206.38 (75.10-564.28)

NPA=Negative Percent Agreement, PPA=Positive Percent Agreement, TPA=Total Percent Agreement **Conclusion:** Our data show good agreement between the results obtained using the novel PMAT assays and the reference methods. Additionally, all analytes in the Aptiva Celiac Disease IgA and IgG Reagents showed excellent clinical performance.

B-064

Development of Non-Competitive Immunoassay for 7S Domain of Collagen Type IV using LUMIPULSE® L2400 Analyzer.

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Background: Collagen type IV is expressed exclusively in basement membranes and its N-terminal noncollagenous 7S domain is more stable than the other domains because of disulfide bonds. In human liver, basement membranes do not exist in the normal perisinusoidal space. Expression of collagen type IV resulting in formation of a perisinusoidal basement membrane increase in early stage of liver fibrosis. Increase of 7S domain of collagen type IV in blood is accompanied by progression of the liver fibrosis. We have developed the fully-automated, novel noncompetitive chemiluminescence immunoassay for LUMIPULSE L2400 analyzer for detecting 7S domain of collagen type IV in serum and plasma, and its performance is evaluated.

Methods: 7S domain of collagen type IV assay using LUMIPULSE systems is a two-step sandwich chemiluminescent enzyme immunoassay (CLEIA). The resulting reaction signals are derived within 30 minutes/sample, and are proportional to the amount of 7S domain of collagen type IV in the sample allowing quantitative determination of 7S domain of collagen type IV in plasma and serum.

Results: The detection limit of the assay was 0.087 ng/mL, and the limit of quantitation was 0.270 ng/mL. A 20-day precision study was performed during a 34-day period using three controls and four panel specimens, and the imprecision was ≤ 2.8% total CV. Linearity was demonstrated over the range 0.9 to 92.6 ng/mL. For spike recovery study, varying amounts of 7S domain of collagen type IV were added to serum samples containing low levels of 7S domain of collagen type IV to create test samples with concentrations ranging from 152.4-550.9 ng/mL. The measured values, when compared to the expected values, ranged from 93-101%. The correlation coefficient and the regression slope of this assay and conventional competitive RIA method (type IV collagen 7S kit, DENIS PHARMA Inc., Tokyo, Japan) were 0.93 and 0.99, respectively (N=133).

Conclusion: Our novel 7S domain of collagen type IV assay using LUMIPULSE L2400 analyzer is well correlated with conventional RIA method and also accurately quantifies low level samples. In addition, this new automated assay can reduce dramatically turnaround time compared to conventional RIA method because of short assay time. This assay is considered as useful for the routine analysis of 7S domain of collagen type IV and the diagnosis for liver fibrosis from large number of chronic liver disease patients.

B-066

Automating Antinuclear Antibody Indirect Immunofluorescence Testing using the EUROPattern System: Comparison to Manual Microscopy

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Background: Antinuclear antibody (ANA) Hep-2 testing via indirect immunofluorescence (IIF) is considered a reference method owing to its high clinical sensitivity. Labor intensiveness, subjective nature of interpretation, and lack of standardization remain as limitations of this methodology. Automated specimen processing coupled with automation-assisted IIF image analysis is expected to increase reproducibility and facilitate standardization of ANA reporting. The objective of this study was to compare the performance of automatically acquired IIF slide image analysis and review *via* a blinded comparison to manual immunofluorescence microscopy. **Methods**: Residual sera (n=127) which represent the titers (1:80 to 1:640) and patterns reported by our laboratory: negative (n=38), homogeneous (n=33), speckled (n=34), nucleolar (n=9), centromere (n=6), multiple nuclear dots (n=4) and cytoplasm (n=3) were utilized. ANA IIF testing was performed using the HEp-20-10 cell substrate using automatic slide processing on the IF Sprinter (Euroimmun AG, Lübeck, Germany). A three-part method comparison was performed between: (1) the standard lab procedure results to (2) automated image acquisition and computer-image review on the EUROpattern (EP) system by 2 skilled medical laboratory technologists (MLTs) and (3) automated results produced by the EP. The standard procedure involved screening (1:80 titer), and titers of up to 1:640, if screen positive. Slides were read manually on a fluorescence microscope (Nikon Eclipse 50i), and the consensus result of 2 MLTs reported. For automated image acquisition, sera were titered at 1:80 and 1:640 dilutions on the IF Sprinter, and processed using the EP system. To facilitate automated image acquisition and review, propidium iodide is incorporated along with the conjugate which allows for nuclear counterstaining and visualization and enhanced EP pattern calling. IIF images were reviewed on the computer screen by 2 MLTs who were blinded to manual microscopy results. EP automated calls were also recorded. For evaluation of comparison results to the standard procedure, up to 1 titer level difference was considered acceptable and agreement for the primary pattern was sought for any given sample. **Results**: The overall agreement for the comparison was determined to be 91%. The negative and positive agreements were 93% and 92%, respectively. In comparison, the EP software produced 72% overall agreement, 70% negative agreement, and 83% positive agreement. The pattern agreement achieved *via* consensus of 2 MLTs was 83% and the titer agreement was 90%. Titer agreements were 90% (n=10), 76% (n=17), 89% (n=28), 94% (n=31) at titers of 1:80, 1:160, 1:320, and 1:640, respectively. For comparison, the EP software resulted in 68% titer agreement. The degree of negative agreement was improved on image review by consensus of two MLTs (93%) versus one MLT (78-80%). Similarly, there was an improvement in

agreements for titer (90% versus 78-84%), and pattern (84% versus 69-66%). There was a pattern-dependent correlation between fluorescence intensity and titer, highlighting the potential for standardization of readings based on intensity and patterns observed. **Conclusions**: Automated slide processing and automation-aided IIF image review has demonstrated comparable performance to manual microscopy in sera of diverse titers and patterns representing the patient population in our laboratory.

B-068

A Comparison Study of Serum Free Light Chain Analysis Performed by Freelite and Diazyme Assays

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Background: The measurement of serum free light chains (FLC) is now the standard of care for patients with monoclonal gammopathies (MG), with inclusion of FLC assessment in the International Myeloma Working Group (IWMG) guidelines. The first commercial FLC assay was Freelite®™, followed more recently by the Diazyme™ assay. Both use polyclonal reagents, however, only Freelite is currently included in the IMWG guidelines. Our goal was to evaluate the Diazyme assay on the Architect c4000 and compare it to the Freelite assay on the Optilite.

Methods: 303 patient samples plus 20 healthy controls were tested using both assays. Samples included 85 MG screens, 45 with a new monoclonal protein (17 IgG/IgA, 24 IgM, 3 biclonal, 1 triclonal), 95 with a known monoclonal protein (71 IgG/IgA, 24IgM), 35 with a light chain only (24κ, 11λ) and 43 AL amyloidosis patients. Passing-Bablok and Bland-Altman analyses were performed. Samples were removed from correlation analysis (9 from κ, 97 from λ) if they were below the stated limits of quantitation (LoQ) of the Diazyme assay. We further assessed the presence of FLC by mass spectrometry on 47 evaluable samples from patients with monoclonal proteins with discrepant FLC ratio (FLCr) results by Diazyme and Freelite for comparison. **Results:** The manufacturer's reference ranges for Freelite were verified in 20 healthy controls. However, 75% of Diazyme λ values were below the LoQ, and so neither the λ nor the FLCr could be verified. We found moderate correlation between assays κ, λ and FLCr using Passing-Bablok and linear regression analysis (y=-1.887+1.121x, R^2=0.80, y=-5.388+0.9361x, R^2=0.74 and y=-0.1051+1.742x, R^2=0.72 respectively). Diazyme λ showed a significant negative bias (-30%), resulting in a significant positive bias for Diazyme FLCr (34%). The under-recovery of λ was seen in all patient groups. Using the recommended reference ranges, the degree of concordance for the patient samples was 90% for κ, 69% for λ and 61% for FLCr. Concordance for all healthy controls was 95% for κ, 45% for λ and 15% for FLCr. Mass spectrometry was 100% concordant with Freelite for determining the FLC isotype in samples with abnormal FLCr by Freelite but normal FLCr by Diazyme. By contrast, MS was concordant with Diazyme in only 45.7% of samples with abnormal FLCr by Diazyme but normal by Freelite. **Conclusion:** There was moderate correlation for all variables between the assays but a poor concordance for λ and FLCr. With 75% of healthy controls and 27% of patient samples having no reliable quantification for λ, the utility of the Diazyme assay for FLCr calculation is questionable and laboratories should calculate their own LoQ for this assay when using the c4000. Mass spectrometry had a stronger agreement with the Freelite assay than Diazyme. A long-term follow up on discrepant results will allow us to assess the clinical significance of the differences between these assays. From these results, we suggest that the two assays cannot be used interchangeably and the Diazyme assay cannot be used when following IMWG guidelines.

B-069

Performance Evaluation of the Complement Factor I Assay for Use on the Binding Site Optilite Turbidimetric Analyser

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Background: The Optilite Complement Factor I (CFI) assay is intended for the quantitative *in vitro* measurement of CFI in serum, lithium heparin plasma and EDTA plasma using the Binding Site Optilite® analyser. CFI is a serine protease regulator of the classical and alternative complement pathways, binding and cleaving membrane-bound or fluid-phase complement proteins such as C3b and C4b to their inactive forms. Measurement of CFI can aid in the diagnosis of CFI deficiency disorder and atypical haemolytic uremic syndrome. Here we describe the performance of an immunoassay for the detection and quantification of CFI on the Optilite® analyser.

Methods: A linearity study was performed (CLSI EP06-A) across the measuring range of 2.50-60.20mg/L. Interference testing was performed (CLSI EP07-A2) at two analyte concentrations using six potential interferents. A comparison study against the Binding Site CFI RID assay was performed using 46 patient samples ranging from 10-49mg/L. A precision study (CLSI EP05-A2) at three analyte concentrations was performed over 20 days using one reagent lot on one analyser. A between analyser study was also performed over 6 days using one reagent lot on three analysers.

Results: The assay demonstrated non-linearity of no higher than ±2.1% when comparing linear and polynomial fits across the curve width. No significant interference effects (±10%) were observed at 10mg/L and 20mg/L CFI when testing unconjugated bilirubin (20mg/dL), conjugated bilirubin (20mg/dL), haemoglobin (200mg/dL), triglyceride (300mg/dL), intralipid (125mg/dL) and ascorbic acid (342µmol/L). Passing-Bablok analysis of the comparison data showed a regression of y=0.8634x+2.63, which reflected the narrow distribution of the patient samples compared to the measuring range. Good precision was observed at all CFI concentrations:

Precision Level	Precision (%CV)				
	Within-run	Between-run	Between-day	Between-analyser	Total
Level 1 – 6.21mg/L	2.0	3.1	1.2	3.1	3.9
Level 2 – 20.61mg/L	1.4	1.8	1.6	3.7	2.8
Level 3 – 39.82mg/L	1.8	2.4	1.4	3.6	3.3

Conclusion: The Complement Factor I assay for the Optilite® provides a reliable and precise method for quantifying Complement Factor I content in human serum, lithium heparin plasma and EDTA plasma.

B-072

Clinical Comparison of Aptiva with Quanta Flash for the Detection of Anti-dsDNA Autoantibodies

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Background: Anti-dsDNA autoantibodies are highly specific for systemic lupus erythematosus (SLE) and are part of the classification criteria. Additionally, depending on the assay used, anti-dsDNA antibodies correlate with disease activity and contribute to the pathogenesis of lupus nephritis (LN). The present study compares Aptiva dsDNA, a particle-based multi-analyte technology (PMAT) immunoassay, to QUANTA Flash (QF) dsDNA chemiluminescent immunoassay (CIA) and their association with disease activity measures. **Methods:** A total of 427 SLE patients, 237 individuals with various other diseases including systemic sclerosis (n=4), rheumatoid arthritis (n=60), primary Sjögren's Syndrome (n=16), idiopathic inflammatory myopathy (n=20), infectious diseases (n=93) as well as 44 apparently healthy individuals were tested for anti-dsDNA antibodies by the novel PMAT (research use only) and by QF dsDNA CIA for method comparison analysis (both methods Inova Diagnostic, USA). Lastly, SLE patients with detailed clinical information available (n=229, patients with or without active disease activity and with or without lupus nephritis) were used to assess the correlation with disease activity and lupus nephritis (using Wilcoxon-Mann-Whitney test). **Results:** Aptiva dsDNA PMAT had a total percent agreement of 82.4% (95% confidence interval, CI 79.8-85.8%) with QF dsDNA CIA, along with 83.5% (95% CI 78.5-87.6%) negative and 82.4% (95% CI 78.4-85.8%) positive percent agreement. The clinical study showed good performance of both assays (details see Table). Additionally, Aptiva dsDNA PMAT and QF dsDNA CIA both showed strong association with disease activity (p=0.0007 and p<0.0001, respectively) and lupus nephritis (p=0.0308 and p=0.0021, respectively).

Characteristic	Aptiva dsDNA PMAT	QF dsDNA CIA
Sensitivity % (95% CI)	63.2 (58.6-67.7)	55.7 (51.0-60.4)
Specificity % (95% CI)	93.7 (89.8-96.1)	92.8 (88.8-95.5)
Likelihood ratio +	10.0 (6.2-16.4)	7.7 (4.9-12.4)
Likelihood ratio -	0.39 (0.34-0.45)	0.48 (0.43-0.53)
Odds Ratio (OR)	25.5 (14.6-44.3)	16.3 (9.6-27.6)
Youden's index	0.57	0.49

Conclusion: Anti-dsDNA antibodies measured using both methods, the novel fully automated Aptiva dsDNA PMAT and QF dsDNA CIA, showed expected clinical performance, good correlation between methods and strong association with disease activity.

B-074

Evaluation of the Anti-Nuclear Antibody interference on the Anti-Neutrophil Cytoplasmic Antibody Indirect Immunofluorescence Assay

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Background: Anti-neutrophil cytoplasmic antibody (ANCA) testing by indirect immunofluorescence assay (IFA) is important for the diagnosis of autoimmune vasculitis. A common analytical interference for the ANCA by IFA is the presence of an antinuclear antibody (ANA), which can cause a seemingly-positive pANCA (perinuclear ANCA) result on ethanol-fixed neutrophils, which may lead to misinterpretation. Laboratories may use ANA results to help guide pANCA interpretation. The purpose of this study was to assess the relationship between the antibody titer, ANA by IFA pattern, or ANA enzyme-linked immunosorbent assay (ELISA) result and pANCA interference.

Methods: Samples (n=317) were collected from ANA by IFA testing which had been ordered as part of routine patient care. Samples collected were negative (n=85) or positive for homogenous (n=60), speckled (n=57), dense fine speckled (DFS) (n=59), or centromere (n=56) patterns, with titers ranging from 1:80-≥1:2560. All samples were undetectable for anti-myeloperoxidase and anti-proteinase 3 antibodies (BioPlex 2200; Bio-Rad, Hercules, CA). All samples were subsequently tested for ANA by ELISA (Bio-Rad). All samples were evaluated for ANCA at a 1:4 dilution on in-house cytospin slides prepared from gradient-purified, ethanol-fixed human neutrophils. Interpretation of the ANCA testing was correlated with ANA ELISA results, ANA by IFA pattern, and antibody titer.

Results: Overall, 10.7% of all samples collected (34/317) resulted in a pANCA interpretation. Of the samples that tested negative by ANA ELISA, 4.7% were interpreted as "pANCA" on ethanol-fixed neutrophils. In comparison, the percentage of pANCA interpretations for samples with ANA ELISA results of 1.0-2.9U, 3.0-5.9U, and ≥6.0U were 22.7%, 13.3%, and 22.0%, respectively. For samples that were negative for ANA by IFA, 4.7% demonstrated a pANCA interpretation. In comparison, 31.7% of samples with a homogenous pattern resulted in a pANCA interpretation, followed by 8.8% for speckled pattern, 6.8% for DFS pattern, and 3.6% for centromere pattern. For samples with an ANA titer between 1:80 and 1:640, 9.7% showed a pANCA pattern, compared to 20.9% of samples with titers ≥1:1280. Of samples with a homogenous pattern at a titer ≥1:1280, 84.6% demonstrated a pANCA pattern on ethanol-fixed neutrophils.

Conclusion: The presence of ANA detected by ELISA was associated with pANCA interference, although this was not correlated with quantitative ANA results. Correlation between ANA by IFA patterns and titers with pANCA interpretations was observed. Of the ANA by IFA patterns evaluated, the homogenous pattern was most frequently associated with pANCA interference, as were titers ≥1:1280. In a majority of cases with high-titer homogenous ANA results, a pANCA interference was observed. Laboratories may take these associations into consideration when using ANA results to clarify pANCA results obtained by IFA using ethanol-fixed neutrophils.

B-075

Validation of Alzheimer's Biomarkers: Amyloid beta 1-40 and Phosphorylated Tau in Cerebrospinal fluid (CSF) by Automated CLEIA on Fujirebio's Lumipulse Platform

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Background: Guidelines 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), β-amyloid 1-40 (Aβ-40), Total Tau (TTau) and Phosphorylated Tau (PTau) proteins have been increasingly included in the diagnostic process of Alzheimer's disease. Aβ-40 peptide is a major component of amyloid deposits. Aβ-40 is used in conjunction with Aβ-42 to determine the amyloid ratio (Aβ-42/ Aβ-40) to aid in diagnosis of AD in patients with cognitive impairment. The combination of decreased concentrations of β-amyloid and increased CSF concentrations of Total Tau and PTau are considered to be pathological CSF biomarker signatures that are used for prognosis of AD. Fujirebio (Fujirebio Inc., Japan) has developed fully automated chemiluminescence enzyme immunoassays (CLEIA) for analysis of Aβ-40 and PTau-181 in CSF along with the existing Aβ-42 and TTau assays. The purpose of this study is to evaluate the performance of the new Aβ-40 and PTau assays as per CLSI guidelines. **Method:** CSF Aβ-40 and PTau 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:** a) Precision: Intra precision: 3 levels of controls tested in replicates of 20 over 1 day, average CV is 1.63 % for Aβ-40 and 2.03 % for PTau Inter precision: 3 levels of controls tested in replicates of 1 over 10 day, average CV is 2.03 % for Aβ-40 and 2.43 % for PTau b) Accuracy: 3 levels manufacturer provided Quality Control and 2 levels of Alzheimer association survey material was tested, all levels are within the target rage range provided by manufacturer and Alzheimer's association for both Aβ-40 and PTau c) Analytical Measuring Range : 5 levels of CSF spiked with recombinant protein covering the target AMR were tested in replicates of 4, AMR of Aβ-40 is 5 to 30,000 pg/mL with slope of 0.986 and AMR of PTau is 1.1 to 400.0 pg/mL with slope of 1.043 d) Sensitivity: 5 levels of diluent spiked with recombinant protein were tested in replicates of 5 per day over 5 days, LLOQ of Aβ-40 is 5 pg/mL with achieved % CV of 10.7% and 1.1 pg/mL for PTau with % CV of 7.4% e) Dilution Verification Two samples diluted with diluent with 2X up to X20, tested in duplicate, dilution acceptable up to X20 for both Aβ-40 and PTau with ULOQ respectively at 600.000 pg/mL and 8.000 pg/mL. f) Length of Run: 3 levels of QC tested over 3 days at three time points during the day (morning, afternoon and evening), No significant change observed throughout the day **Conclusion:** Lumipulse G Aβ-40 and PTau are robust quantitative assays and meet the CLSI requirements. CSF Aβ-40 and PTau could be proposed in clinical or drug trials as markers for AD according to the guideline. **References:** 1. Alzheimer's Dement. 2011, 7(3): 257-262, 2. Alzheimer's and Dementia, 2011, 7, 3, 280-292 2. Alzheimer's and Dementia, 2011, 7(3): 270-279, 4. Alzheimer's and Dementia, 2011, 7, 3, 263-269

B-076

Precision Evaluation, Bias Estimation and Sigma Metrics in Different Immunoassays Profiles of the Atellica IM 1600 Analyzer

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Introduction: Most clinical decisions in patient management are made based on the results of laboratory tests. Therefore, it is essential that the clinical laboratory adopts measures to mitigate risks to patient safety, implementing quality control processes. Quality management with Six Sigma metrics is used worldwide for assessing the capacity of a process. When applied to the clinical laboratory, this methodology can demonstrate how much the test can generate results out of its quality specifications. Thus, the study was based on performance evaluation through studies of imprecision, bias estimation and sigma metrics analysis of 23 different clinical profiles immunoassays during the implantation of Atellica IM in a laboratory in São Paulo, Brazil. **Methods:** The precision study was performed with each level of QC tested in one run per day, with five replicates per run for five days, resulting in a total of 25 replicates per sample for each trial. The repeatability study ($\%CV_R$) and within-laboratory imprecision ($\%CV_{WL}$) was performed in accordance with EP15-A3 for the imprecision evaluation and bias estimation by peer groups. Sigma metrics were calculated for each assay using the bias and the precision study for each level of QC. The first choice of Total Allowable Error (TEa) specification for the proposed study was based on the 2014 Biological Variation and RiliBÄEK table, in this priority order. **Results:** The results agree with those with the analytical quality specifications. The $\%CV_{WL}$ obtained was 1.37% to 10.43% and the $\%CV_R$ was 1,07% to 5,07% among all assay levels tested in the Atellica IM Analyzer. The evaluation of sigma metrics was performed by clinical profiles for Anemia, Bone Metabolism, Diabetes & Metabolic Diseases, Tumor Markers and Fertility, obtaining approximately 16.7%, 75.0%, 83.3%, 75.0% and 27.7% of the results, respectively, with six sigma performance (world class). All tests obtained acceptable performance with sigma above 3. **Conclusion**: All tests demonstrated acceptable results of precision and sigma, consistent with the analytical quality specifications. Sigma metrics can be incorporated as another form of periodic performance evaluation, mainly after understanding the assays performance in the new platform implantation. However, it is important to be aware of the limitations that must be considered when interpreting the results, such as the lack of TEa targets for some analytes, leading to the use of different specifications for the same analysis and the same test profile. **Siemens Healthineers supported the studies by providing systems, and reagents.*

B-077

Performance Evaluation of the Abbott ARCHITECT and Alinity i Thyroglobulin (Tg) Chemiluminescent Microparticle Immunoassay

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Background: Thyroglobulin (Tg) is a 660 kDa glycoprotein exclusively produced by the thyroid gland. Measurement of Tg is an essential element of the follow-up and management of patients with differentiated thyroid cancer (DTC). The concentration of Tg in the blood reflects thyroid mass, thyroid injury and TSH receptor stimulation. Tg detection in treated DTC patients has proven clinically useful with even very low amounts indicating persistence or recurrence of the disease. The capability to detect very low concentrations is achieved by second-generation immunometric Tg assays with a functional sensitivity of <= 0.1 ug/L, which allows early detection of relapse without the need for TSH stimulation assays, which had to be performed for less sensitive first-generation assays. **Objective:**1)Demonstrate the analytical performance of the ARCHITECT Tg and Alinity i Tg assays 2)Evaluate the analytical performance of the ARCHITECT Tg assay in a hospital laboratory, comparing the new Abbott ARCHITECT and Alinity i second-generation Tg assays with the Roche Tg II assay. **Methods**:Testing included assessments for precision, limit of quantitation (LoQ) and method comparison. At the University Health Network in Toronto, precision was verified using a five-day precision protocol per CLSI EP5A3 guideline and the LoQ was verified with 3 different plasma pools. Serum samples were used to measure Tg and anti-Tg on Abbott ARCHITECT i2000SR and Roche Tg II using the cobas e 411 analyzer. Samples negative for ARCHITECT anti-Tg were selected to represent the whole ARCHITECT Tg measurement range. In addition, specimens positive for anti-Tg were collected and Tg was measured on ARCHITECT, Alinity i and cobas e 411 platforms. **Results:**The observed results for precision, LoQ and method comparison obtained at Abbott Laboratories and at UHN are shown in the table below.

Evaluation	Abbott Alinity	Abbott ARCHITECT	UHN ARCHITECT
Precision (Total %CV)	1.5 to 8.8 % Sample range: 0.20 to 434.30 ng/mL	1.6 to 9.3 %Sample range: 0.17 to 435.80 ng/mL	1.7 to 8.1 %Sample range: 0.19 to 337.82 ng/mL
Method Comparison vs Roche Tg II (Slope/ Correlation)	0.99/0.98Range: 0.15 to 476.69 ng/mL	1.04/0.99Range: 0.18 to 473.97 ng/mL	0.98/0.98Range: 0.10 to 464.9 ng/mL
LoQ (Verified)	0.09 ng/mL	0.10 ng/mL i1000SR0.09 ng/mL i2000SR	0.09 ng/mL

Conclusion: Acceptable performance for precision, method comparison and sensitivity were obtained, and agreement was observed between the manufacturer verification results and the results at a customer site.

B-081

Evaluation of the MAGLUMI 2000 Chemiluminescent Anti-dsDNA Immunoassay

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Background: Autoantibodies to double-stranded DNA (anti-dsDNA) is useful for the diagnosis and monitoring of patients with systemic lupus erythematosus (SLE). Maglumi 2000 (Snibe, Shenzhen, China) is an automated chemiluminescent immunoassay system with a broad menu of tests. Recently, Snibe introduced a rapid assay for anti-dsDNA. **Methods:** In the Snibe anti-dsDNA assay, samples, buffer and magnetic microbeads coated with dsDNA antigen are thoroughly mixed at 37°C. When a magnetic field is applied, materials bound to the microbead are separated from unbound materials which are then washed away. Thereafter, mouse anti-human IgG labelled with N-(4-aminobutyl)-N-ethylisoluminol (ABEI) is added to form a sandwich complex. After another application of magnet and wash, followed by a starter solution, a chemiluminescent signal is generated. The light signal is proportional to the anti-dsDNA concentration in the sample. The assay is standardised against the WHO 1st International Standard Wo/80. Results are determined from a 2-point calibration curve with each assay and a 10-point master calibration curve with each reagent lot. Manufacturer suggested cut-off for healthy subjects is 30 IU/mL. **Results:** The Snibe Maglumi 2000 is easy to operate with minimal daily maintenance. The inter-assay precision (CV%) of the 2 levels of kit controls are 2.3% at 22 IU/mL and 2.6% at 203 IU/mL of anti-dsDNA. The assay was verified to be linear from 1.7-768 IU/mL (claimed 1-800). The limit of quantitation (LoQ) of this assay corresponding to a CV of 20% was found to be 2.6 IU/mL (claimed 1.5). Assay time is approximately 30 mins. We also evaluated the performance of the Snibe anti-dsDNA compared to an established automated enzyme immunoassay (Bio-Rad, USA) at a referral laboratory. In 64 healthy subjects, 62 were concordantly negative in both assays. However, the remaining 2 subjects were reported as weak positive on the BioRad while the Snibe gave very normal results - 3.7 and 14.5 IU/mL. In 25 patients with SLE, both assays reported positive anti-dsDNA in 19 subjects. It is unclear if the remaining 6 patients are true positive, in remission or are anti-dsDNA negative. Bio-Rad classified 4 of them as weak positive (Snibe reported negative values - anti-dsDNA results ranged from 3.0-23.0 IU/mL), 1 as positive (Snibe - 17 IU/mL) and 1 as strong positive (Snibe 24 IU/mL). **Conclusion:** The performance claims of the Snibe anti-dsDNA are in line with the manufacturer's claims. The Snibe assay is attractive because of its ease of operations, rapid TAT (compared to 3 hours for Bio-Rad) and more quantitative result reporting.

B-083

Analytical Performance and Clinical Accuracy of Serum Free Light Chains Assays

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Background: The free light chains monoclonal immunoglobulin (kappa- SFLCK and lambda-SFLCL free light chains) are the important tumor markers in serum and urine in the diagnosis and prognosis of patients with monoclonal diseases of plasma cells .The validation procedures ensure that the analytical methods are accurate, reproducible, robust in a specific range in the analytes will be analyzed, according compliance with the requirements of good practices in clinical laboratory and patients safety. This study was done to characterize the analytical performance and clinical accuracy of a new equipment OPTLT for SFLCK and SFLCL measurements, comparing with the system in use (c502), serum protein electrophoresis, immunofixation and K / L ratio. **Methods:** This study was carried out in a public Brazilian tertiary hospital. The analytical performance of OPLT was analyzed following the manufacturer's instructions. 20 samples were analyzed in the OPTLT, including low L, intermediate I and high H sera The results were compared with those produced by the Cobas c502 . The evaluation envolved: lower limit of quantification, carryover, robustness, between run precision, within run precision, and verification of linearity , reference ranges. Clinical accuracy: OPTLT X serum Electrophoresis, Immunofixation and Relation kappa/lambda in 100 patients with diagnosis of monoclonal gammopathy were compared. The level of agreement, false positives and false negatives between the methods was evaluated. 100 samples of serum from outpatients for 20 consecutive days were used to the study, including male and female with ages between 30 - 78 years old. True positive, false positive, true negative and false negative results were selected. The area under the ROC curve for SFLCK and SFLCL was used, calculating the percentage of clinical accuracy, specificity, sensitivity, and cut-off. Statistical analysis: descriptive statistics, regression analysis, Pearson correlation coefficient, coefficient of determination, error index, receiver operating characteristic curve- ROC. **Results:** Within run precision (L-I-H):adequate10%- SFLCK(%):5.7-2.9-3.0 SFLCL(%):7.0-3.3-3.5. Between run precision(L-I-H): adequate10%- SFLCK(%): 6.6-3.4-3.9 SFLCL(%):8.5-4.0-4.5 Carryover adequate; 95 Percentil Range: SFLCK: 3,30-19,40 mg/L SFLCL:5,70-26,30 mg/L Total range to Relation K/L: 0,26-1,65 ; AMR: SFLCK: 2.6-140.0 mg/L SFLCL: 4.1-150 mg/L; CRR: SFLCK: less then2.6 and greater then 140.0 mg/L SFLCL: less then 4.1 and greater than 150 mg/L SFLCK c502= - 2.337 + 1.0701 OPTLT r=0.997 p=0.000 R^2=99,31% SFLCL c502=0.91+0.8919 OPTLT r=0.997 p=0.000 R^2=99.44% CLINICAL ACCURACY: It was observed that the Kappa / Lambda ratio is useful in the diagnosis of monoclonal gammopathies, but it presents false positives (samples with hypergamma globulinemia). There was agreement between results of electrophoresis of serum proteins, immunofixation,SFLCK and SFLCL. The SFLCK and SFLCL presented good results of specificity, sensitivity, and percentage of clinical accuracy. **Conclusion:** The two analytical system(SFLC Kappa and SFLC Lambda using OPTLT) showed good performance. The comparison with the analytical system in use in the laboratory (c502) demonstrated excellent correlation(r=0.997 and R^2 greater than 99%).There were good correlation of SFLCK and SFLCL with serum Electrophoresis, Immunofixation and relation kappa/lambda from already diagnosed patients and outpatients, demonstrating its clinical utility in the diagnosis and monitoring of monoclonal gammopathies.

B-084

Performance of Immunotyping Versus Immunofixation for the Detection of Monoclonal Immunoglobulins

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Background: Serum immunofixation (IF) is routinely used to detect and isotype monoclonal immunoglobulins (M-proteins). This is an important component of the International Myeloma Working Group guidelines for monitoring patient response to treatment. However, IF is a highly manual, low-throughput assay. Immunotyping (IT) by capillary electrophoresis offers several workflow advantages such as higher throughput, automated analysis, and digital results. We hypothesize that technical advances and interpretive training may affect the performance of IT compared to what has been previously reported. The objective of this study was to evaluate the analytical performance of IT compared to IF in a population with a high prevalence of monoclonal gammopathies.

Methods: Waste clinical serum samples (n=1000) were obtained from the clinical laboratory at Memorial Sloan Kettering Cancer Center (MSKCC). Specimens were collected over a 1-month period and included any sample that had serum protein electrophoresis (SPEP), IF and free light chain studies ordered for clinical purposes. Samples were analyzed within 5 days of collection on a CAPILLARYS 3 Tera instrument (Sebia, Inc). IT results were interpreted by 3 experienced reviewers from the Result Interpretation Escalation Team at Sebia. Reviewers were blinded to IF results and clinical history. IF results were interpreted by MSKCC attendings who were blind to IT results and clinical history. For both IT and IF, a consensus agreement was reached when individual interpretations were discordant. To compare the techniques, results were categorized as either normal (no M-protein present) or abnormal (monoclonal, biclonal, or multiple bands present). We also determined the M-protein detection rate of 2 different test panels: Panel A (SPEP, free light chains and IF) and Panel B (SPEP, free light chains and IT). A sample was considered positive if any test in the panel was abnormal. Finally, interpreter agreement within each technique was evaluated.

Results: IT results were concordant with IF in 87% of samples. 698 samples (70%) were positive for an M-protein by both methods and 172 (17%) samples were negative for an M-protein by both methods. For the 13% of samples that were discordant, 74 samples (7%) were positive by IT only and 56 samples (6%) were positive by IF only. All discordant cases occurred in samples that did not have an apparent M-spike on SPEP. For all SPEP-positive samples (n=573), IT was concordant with IF. We found that 843 samples were positive by Panel A (SPEP/IF/FLC) and 865 samples were positive by Panel B (SPEP/IT/FLC). Reviewer discordance within a test (26% and 22% for IF and IT, respectively) was greater than the discordance between IT and IF (13%).

Conclusion: IT provides equivalent results to IF for the detection of monoclonal proteins. IT was concordant with IF in 100% of SPEP-positive samples. At lower concentrations when an apparent M-spike is not visible by SPEP, neither IT nor IF provided a conclusive result regarding the presence or absence of an M-protein. Further investigation is needed to determine whether M-protein(s) are present in these samples. The clinical significance of these possible low-level M-proteins is also under investigation.

B-085

Evaluation of Kappa Index as a Tool in the Diagnosis of Multiple Sclerosis

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Introduction: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Leurs CE, *et al.* (Mult. Scler. May 2019) have shown that cerebrospinal fluid Kappa-free light chains (CSF-K FLC) have higher sensitivity and slightly lower specificity than the gold standard OCB (oligoclonal bands) for the detection of intrathecal immunoglobulin synthesis, a characteristic feature of MS. CSF-K offers a quantitative and easy to standardize method for MS diagnosis, but a definite cut-off still has to be established to be routinely used.**Objective:** evaluate the performance of the kappa index (KI=CSF-K/serum-K FLC divided by CSF/serum albumin) for differential diagnosis of MS. **Methods:** 120 patients were included: control group, containing patients with dementia, stroke, and non-demyelinating CNS inflammatory diseases (n=99); and, patients with confirmed MS (n=21). K-FLC were measured, in parallel serum and CSF samples, by turbidimetry (Freelite on Optilite, Binding Site). 82 (68,3%) patients had CSF-K<0,03mg/dL, below the sensibility limit of the technique, and none of them had a diagnosis consistent with MS. Leurs *et al.* previously described a CSF-K mean value of 0,020mg/dL in no-MS population, so, in order to calculate the KI we considered CSF-K=0,020mg/dL whenever it was <0,03mg/dL. ROC analysis was preformed by GraphPad Prism8. **Results:** MS had higher KI (mean:90,39; min:7,08; max:471,0) than no-MS patients (mean:7,828; min:0,93; max:95,0). Results (Table 1) applying previous cut-off of KI>6,6 confirm a higher sensitivity and lower specificity than OCB (Leurs etal,2019: sens. 93%, spec. 83%). With 100% sensitivity, this cut-off could be helpful has a first screening technique to rule out MS and maybe afford further OCB studies (68/120 samples, 56,7%). A KI cut-off of 13,87 would compare well to the performance of OCB.

Conclusions: Our findings validate confirm the KI diagnostic performance on a turbidimetric analyzer. KI may also help to optimize the diagnostic protocol.

	BOC	KI>6,6	KI >13,87
P value	<0,0001	<0,0001	<0,0001
Sensitivity	**0,9524**	**1**	**0,9524**
Specificity	**0,899**	**0,6869**	**0,899**
Positive Predictive Value	**0,6667**	**0,4038**	**0,6667**
Negative Predictive Value	**0,9889**	**1**	**0,9889**
Likelihood Ratio	9,429	3,194	9,429

B-086

Validation of Fecal Calprotectin (fCal®turbo) by an Automated Routine Chemistry Analyzer Architect C 4000 Platform

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Background: Guideline for Inflammatory bowel disease (IBD) suggests using Fecal calprotectin (FC) for patient during diagnostic and monitoring phases, as well as in distinguishing between IBD and IBS (Irritable BowelDisease). Crohn's disease and Ulcerative Colitis are the principle types of Inflammatory Bowel disease. Fecal Calprotectin (FC) is a heterodimeric protein (S100A8/A9) released by granulocytes, monocytes,macrophages and epithelial cells during inflammatory response, with inflammatory cells accumulating inmucosa. FC is released into the stool, from where is can be measured using commercially available assays.Bühlman (Bühlman Laboratories AG, Switzerland) has developed a fully automated turbidimetricimmunoassay for calprotectin analysis. The purpose of this study is to evaluate the performance of thisassay as per CLSI guidelines and compare the results of the assay with Bühlmann ELISA.**Method:** Fecal calprotectin (S100A8/A9) is measured quantitatively by particle enhanced turbidimetricimmunoassay (PETIA) method on the Architect c4000 (Abbott Diagnostic, US) using immunoreaction cartridges. **Results:**

Validation parameter	Experimental design	Results fcal®turbo	Results ELISA
Precision	**Intra Assay:** 3 levels, 20 replicates each, 1 day	Average % CV= 0.56 %	Average % CV= 7.36
Precision	**Inter Assay:** 3 levels, 1 replicate per day, 10 days	Average % CV= 0.54 %	Average % CV= 9.83
Accuracy	**- CAP* proficiency**: 6 samples **-2 kit controls**	All samples were within CAP* ranges. Avg recovery: 97%	All samples were acceptable by CAP*
Analytical Measuring Range	5 levels of stools freshly extracted, 4 replicates each, 1 day	25 to 1800 µg/g, slope = 0.988	25 to 1800 µg/g , slope = 0.940
Sensitivity (LLOQ)	5 levels, stools freshly extracted, 5 replicates each per day, 5 days.	27.4 µg/g, CV = 7.5 %	21.9 µg/g, CV = 12.9 %
Dilution Verification	Two stool samples diluted with diluent with 2 fold dilution tested in duplicate up to X20 automatically.	Automated dilution X20 is acceptable. ULOQ = 36000 µg/g,	Manual dilution x16 ULOQ = 28800 µg/g
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 plate
Interference	Hemolysis, Total bilirubin, Triglycerides evaluate on the freshly extracted stools per CLSI guideline C-56A.	Hemolysis: 21.67 mg/dL Triglycer.: 220.3 mg/dL Total Bili: 0.21 mg/dL.	Hemolysis: 50 mg/dL
Correlation instrument vs ELISA	40 freshly extracted stool samples tested on the Architect c4000 and by ELISA in parallel X method: ELISA Y method: Architect c4000	**Slope** = 0.957 **Intercept** = 4.47 **Corr coef (R)** = 0.9928 X Mean = 320 µg/g Y Mean = 311 µg/g	

*CAP: College of American Pathologist **Conclusion:** The Fecal calprotectin fcal®turbo on Architect c4000 is a robust quantitative assay and may meet the Clinical and Laboratory Standard Institute (CLSI) requirements. Fecal calprotectin by PETIA could beproposed in clinical or drug trials as markers for IBD accordingly to the guideline.

B-087

Alteration in Laboratory Tests and Multiple Myeloma Diagnosis

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Background: Multiple myeloma (MM), a neoplasm of plasma cells, is characterized by abnormal clonal proliferation of plasmocytes in the bone marrow, with production of monoclonal immunoglobulins, associated with organic dysfunction and can lead to the compromise of vital organs, lytic lesions, renal failure and malaise immune system performance. MM represents 1% of neoplastic diseases and 13% of hematological neoplasms, being responsible for 10% of deaths caused by hematological cancer in the world. In Brazil, there is no exact knowledge of the incidence of this disease. Often, the disease does not cause symptoms until it reaches an advanced stage. At other times, it can cause vague symptoms that can be mistaken for other diseases. One of the first laboratory changes observed in the diagnosis of MM is the monoclonal production of immunoglobulins, by tumor cells. The most used laboratory tests to identify and quantify these immunoglobulins are serum protein electrophoresis and immunofixation. The first is a screening method that will measure the total amount of circulating immunoglobulin and show the presence of any abnormal immunoglobulin. The second is a method that determines the exact type of immunoglobulin being produced (IgA, IgM, IgG or some other type). **Objective**: To describe the epidemiological characteristics of MM in Brazil during the last three moths. **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 serum electrophoresis and immunofixation obtained and released from November 2019 to January 2020 were compiled. Epidemiological data such as gender, age and region of the country of patients were statistically analyzed. **Results:** A total of 9419 patients were evaluated between November 2019 to January 2020, which 42.2% were male and 57.8% were female. The most part of patients were from Southeast region (52.8%) and most of them were between 61 and 70 years old (25.7%). 46.3% of patients had an alteration results in immunofixation tests but in 72.9% of these patients did not perform the electrophoresis test. Of the 1,183 patients who also underwent electrophoresis, 76.8% had hypergammaglobulinemia, with monoclonal gammaglobulin peaks being observed with their median concentration around 1.27 g/dL (the maximum concentration being 9.26 g/dL) and their median percentage in relation to the total proteins of the patients 19.1% (the maximum percentage was 77.2%). **Conclusions**: In view of the lack of data on the Brazilian population, this study shows that 9.6% of patients who seek the diagnosis of MM through electrophoresis and immunofixation tests have hypergammaglobulinemia and some type of monoclonal protein, which is in accordance with the evidence in the literature . MM is most often preceded by precursor stages of monoclonal gammopathy of undetermined significance (MGUS) and by latent MM. The results gives us a glimmer of the profile of patients with MM in Brazil, more studies are necessary to define this profile.

B-088

Retrospective Validation of the APE^2 Score in Autoimmune Encephalopathy Evaluation Panels

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Introduction: Autoimmune encephalitis is a debilitating condition with a variety of underlying causes. Many reference laboratories offer testing for antibodies associated with autoimmune encephalitis in serum and CSF. However, many of the symptoms, such as confusion, seizure activity and autonomic dysfunction are not specific to autoimmune encephalopathy. To help identify patients likely to have an autoimmune cause, Dubey and colleagues recently published a scoring system known as the Antibody Prevalence in Epilepsy and Encephalopathy (APE^2) score (1). We sought to retrospectively examine the effectiveness of the APE^2 scoring system in autoimmune encephalopathy evaluations that had been sent from Dartmouth Hitchcock Medical Center (DHMC).

Methods: Results from 286 (139 CSF and 147 serum) patient autoimmune encephalopathy evaluation panels sent for testing between 10/9/2016-10/12/2019 were evaluated in the context of the patient's medical record and presence of antibody above the reference interval. Panels were classified as, "true positive," if a diagnosis or change of care was made as a result of the panel. The APE^2 score was applied to all panels with at least one antibody above the lab-determined positive threshold, and to date, 32 negative panels between 01/1/2019-06/12/2019. An APE^2 score of 4 or more indicated that an encephalopathy panel was warranted, with scoring criteria previously listed (1).

Results: Of the 286 panels, 32 (11%) were positive for any antibody; 4 (1%) being CSF and 28 (10%) serum. Within the positives panels, 8 (3%) were determined to be true positives (3 CSF, and 5 Serum). The true positives had a median APE^2 score of 5 (IQR:2.25-6.75) with 3 having an APE^2 score <4 and 5 with a score of ≥4. Positive panels not clinically acted upon had a median APE^2 score of 3 (IQR:2-4), with 13 having an APE^2 score <4 and 5 with a score of ≥4. The 32 negative panels had a median APE^2 score of 2 (IQR:1-3), with 28 having a score of <4, and 4 ≥4.

Conclusion: The majority (88%) of negative panels, those not positive for any antibody in either serum or CSF, would not have been ordered had the APE^2 score been used as a determining factor. In addition, while 17 (53%) of all positive encephalopathy panels would not have been ordered had the APE^2 score been implemented, 14 (82%) of those would not have been clinically acted upon. Conversely, only 3 (37.5%) of true positive encephalopathy panels would not have had been ordered given their APE^2 score. Overall, inclusion of the APE^2 score has the potential to improve the diagnostic utility of the autoimmune encephalopathy evaluation panels.

1.Dubey, Divyanshu, et al. "Predictors of neural-specific autoantibodies and immunotherapy response in patients with cognitive dysfunction." *Journal of neuroimmunology* 323 (2018): 62-72.

B-089

Comparison of Trypsin Enzymes for Simultaneous Calprotectin and Lactoferrin Detection in Stool Samples by LC-MS/MS

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Background: Fecal calprotectin (FC) and lactoferrin (FL) biomarkers have been emerged as a noninvasive diagnostic tool to distinguish inflammatory bowel disease (IBD) from other functional gastrointestinal disorders, such as irritable bowel syndrome. Simultaneous quantification of these fecal biomarkers using a peptide-based LC-MS/MS method could have advantages compared to conventional immunoassays. However, initial development demonstrated unacceptable imprecision within LC-MS/MS extraction method. We hypothesized that uncontrolled proteolysis from endogenous proteases could be altering the peptides produced from the trypsin digestion. The objective of this study was to determine if thermally-stable trypsin improved

the intra-assay precision of FC and FL detection in stool samples using LC-MS/MS. ***Methods:*** Eight residual stool samples with calprotectin values between 22-434 ug/g as quantified by QUANTA Lite™ Calprotectin ELISA (Inova, San Diego, CA) were used. The FC and FL were extracted by vortexing with ceramic beads in a solution of 17% formic acid, 33% isopropyl alcohol, and 50% water, followed by a neutralization step with NaOH and addition of winged internal standards. One conventional trypsin enzyme (Worthington) and two proprietary, thermally-stable trypsin digestion kits (Thermo and Promega) were evaluated. The extraction for digestion by the Worthington enzyme was reduced with dithiothreitol, followed by alkylation with iodoacetamide; extractions for the Thermo and Promega enzymes did not require additional processing. Extractions were digested for 2h at 37°C (Worthington) or 1h at 70°C (Thermo and Promega). A total of four targeted peptides identified during a discovery phase were used: one peptide for each calprotectin subunit (S100A8-ALN and S100A9-LGH); and two peptides for lactoferrin (Lacto-LRP and Lacto-EDA). Five replicates of each sample extraction were digested with each enzyme. A targeted LC-MS/MS method was used for simultaneous relative quantification of calprotectin and lactoferrin peptides using a SCIEX Triple Quadrupole 6500 Mass Spectrometer System (Framingham, MA U.S). The variance of the corrected peak intensity of each peptide signal was used to evaluate analytical imprecision, which was expressed as percent coefficient of variation (%CV). ***Results:*** The intra-assay precision was calculated for trypsin digestion for 4 peptides from calprotectin and lactoferrin in 8 patient samples, for a total of 32 measurements for each peptide. The average %CV of the intra-assay precision across the four peptides for the Worthington, Thermo and Promega trypsin enzymes was 12%, 7% and 6%, respectively. However, despite the acceptable average %CV, the Worthington enzyme demonstrated %CV of 11-20% in 8 measurements and %CV>30% in 4 measurements, with only 20 measurements having %CVs≤10%. In comparison, the Thermo and Promega trypsins showed only 6 and 3 measurements with CVs between 11-20%, respectively, with all other measurements having %CVs <10%. ***Conclusion:*** Thermally-stable trypsin offered improvements in reproducibility compared to regular trypsin for a simultaneous detection of targeted peptides of FC and FL in stool samples. This work provides the foundation for further optimization of FC and FL quantitation by LC-MS/MS, and may possibly be expanded to other stool biomarkers.

B-090

Measles Serology Response to Mumps, Measles and Rubella (MMR) Vaccination in Health Professionals from Brazil

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Background: Measles is an acute viral disease, highly transmissible by aerosols, directly from person to person through nasopharyngeal secretions by the infected subject. After being considered out of circulation in Brazil in 2016, the disease returned in the country in 2018. This has been the target of frequent public vaccination campaigns. According to the Centers for Disease Control (CDC), one of the following is accepted as evidence of presumptive measles immunity: a) adequate vaccination documentation (one or more doses of measles component vaccine administered after the first year of life for preschoolers and adults not considered at risk) or two doses of vaccine with measles component administered to school children and high-risk adults including university students, health professionals and international travelers); b) laboratory evidence of immunity; c) laboratory confirmation of measles; d) birth before 1957. This study demonstrates the characteristics of measles serology in a group of health professionals undergoing vaccination by the MMR.

Methods: This study includes 75 health professionals who were vaccinated with a dose of the MMR vaccine (Bio-Manguinhos/Fiocruz), according to the national vaccination protocol. Serum samples were collected at day 0 (D0) (before vaccination) and day 28 (D28) (post vaccination), for analysis of IgM and IgG for measles. The serologies were performed by chemiluminescence. **Results:** At D0, of the 75 individuals tested, 11 individuals had IgG serology for measles other than reagent (10 non-reagent (NR) and 1 indeterminate (I)). Of these, 10 individuals presented IgG reagent at D28, with 1 individual before NR converted I. This individual was followed up after 3 months of vaccine administration, when demonstrated NR results. This case was a puerperal woman with a previous history of 2 doses of MMR vaccine. Regarding IgM status, all 75 individuals were NR at D0, and 1 case converted reagent at D28, besides being previously immune to measles at D0 (IgG reagent).

Conclusion: Although small sampling, the present study demonstrates that the complete vaccination schedule with 2 doses of MMR is not necessarily reflected in an IgG serology reagent for measles. In addition, previously immune individuals can again demonstrate IgM reagent in the context of a new dose of vaccination.

B-091

Positivity of High and Low Risk Human Papillomavirus (HPV) in Male Samples under the Molecular Technique of Hybrid Capture

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Background: The human papillomavirus (HPV) in men, causes an infection that, in general, is incipient, without clinical manifestations, which impairs the individual's diagnosis, making him an asymptomatic carrier and active disseminator of this virus. This characteristic contributes to female contamination by 70%, due to the fact that their partners have HPV. Currently, there are more than 200 types of HPV described in the literature, divided into high and low risk according to their oncogenic potential. In this study, the authors evaluated the positivity for high and low risk HPV in male samples submitted to analysis by hybrid capture, and related to the age range and topography of the material. Methods: The study consisted of an analytical, observational and retrospective review. Considered were only male patients, without age limits, with a request for HPV research by hybrid capture for only one sample. The patients were divided into groups, according to the age, for better analysis, as well as the topographies. The method used was the molecular technique of Hybrid Capture II (CHII), in the Rapid Capture System (RCS) equipment - USA, with digene HC2 HPV DNA test kit - QIAGEN®. The results were evaluated and stratified into four groups, considering high risk and low risk: (HR + / LR +), (HR + / LR-), (HR- / LR +) and (HR- / LR-). Data were obtained from the system of a large laboratory in the city of São Paulo. Results: A total of 1,868 patients were analyzed, aged 6 months to 73 years. 68.62% presented an HR- / LR- result (n = 1,282), 12.32% with an HR- / LR + result (n = 230), 10.54% with an HR + / LR + result (n = 197) and 8, 52% resulted in HR + / LR- (n = 159). One-way ANOVA testing was performed between the groups, with no significant difference between them (p> 0.05), except when compared with the HR- / LR- case group (p = 0.0002). When compared to the age group, it was observed that 26.55% were from patients aged 26 to 30 years (n = 496), of these, 18.34% (n = 91) of the cases were HR+, and 12, 29%, (n = 61), LR+. The numbers call attention to patients who presented HPV HR +, 17.64% (n = 9) aged <20 years. About topographies, the largest amount of materials sent was from the penis (considered as only body and base). Glans and foreskin were analyzed separately. 79.87% (n = 1492) were analyzes of penis samples, of which 19.83% (n = 296) HR+; urethra with 6.37% (n = 119), of which 10.92% (n = 13) were positive for HR. Conclusion: The study showed high positivity of HR in young men <20 years old. The dominant topography was in penile injury. Due to asymptomatic men, the study highlighted the importance of raising awareness of vaccination in young men and women. The importance of using molecular methods as major allies in the detection of HPV before oncogenic evolution is also emphasized.

B-092

Comparison of Two Different Multiplex Specific IgE Detection Immunoassays: PROTIA™ Allergy Q and Advansure™ AlloScreen

A. Lee, J. Jang. *Seoul Clinical Laboratories, Yong-In, Korea, Republic of*

Background: The identification of clinically meaningful specific immunoglobulin E (sIgE) is essential for the diagnosis and management of various allergic diseases. The multiplex sIgE immunoassay method is increasingly used as a screening tool for allergic diseases in Korea recently. This study aimed to compare two different multiplex sIgE immunoassays, both of which have been developed and popularly used in Korea. **Methods:** Remnant sera of 78 patients for whom the multiplex sIgE assays were requested were collected. PROTIA™ Allergy Q (ProteomeTech, Seoul, Korea) and Advansure™ AlloScreen (LG Life Science, Seoul, Korea), both of which are based on the enzyme immunoassay technique that uses membrane stripe as the solid phase for allergen immobilization, were compared. The concentrations of sIgEs to each allergen in two multiplex assays were categorized from class 0 to 6. Results higher than class 1 were defined as positive. Results of two or more class difference between two assays for each allergen were regarded as disagreement. The ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) sIgE single-plex assay for selective antigens was performed to resolve discrepant results between two multiplex sIgE immunoassays. **Results:** In qualitative comparisons, the positivity and negativity agreements between

two multiplex sIgE immunoassays showed overall 91.8% agreement (kappa=0.71) for 81 matched allergens. Agreement rates for each allergen ranged from 66.4% to 100.0%. Qualitative agreement for total IgE (cut-off, 100 IU/mL) between two multiplex sIgE assays was 82.1%. **Conclusion:** The multiplex sIgE detection immunoassay method is a powerful tool for its simultaneous multiple allergens detecting capability with small sample volume in short assay time. Although many allergens showed good agreement, some allergens showed modest agreement between two different multiplex sIgE assay systems.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Hematology/Coagulation

B-095

Early Pre-Microscopic Differentiation of Acute Promyelocytic Leukemia; the Convergence of Cell Population Data and Artificial Intelligence

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Background: A targeted and timely offered treatment can be a benefitting tool for patients with hematological emergency like acute promyelocytic leukemia (APML). Complete blood count (CBC) is a key test in diagnostic workup of hematological neoplasms, for which modern hematological analyzers generate cellular measurements as cell population data (CPD) in background for reporting blood cell's counts. We hypothesized that pre-microscopic flagging of "acute leukemia (myeloid/ lymphoid) of analyzed blood sample by the hematology analyzers could be life saving. Such a flagging will alert the technologist to bring the slide of the flagged sample to the hematologist for early review.

Methods: Using artificial intelligence (AI) algorithm and based on CPD parameters, we have built artificial neural network (ANN) model to early flag of (predict a) APML. We collected classical CBC items along with cell population data (CPD) from hematology analyzer at diagnosis of 1067 study subjects with hematological neoplasms. For morphological assessment peripheral blood films were examined. Statistical and machine learning (ML) tools including principle component analysis (PCA) helped in the evaluation of predictive capacity of routine and CPD items. Then selected classical CBC and CPD parameters driven ANN predictive modeling was developed to smartly use the hidden trend by increasing the auguring accuracy of these parameters in differentiation of APML cases.

Results: We found a characteristic triad based on lower (53.73) platelet count (PLT) with decreased/normal (4.72) immature fraction of platelet (IPF) with addition of significantly higher value (65.5) of DNA / RNA content related Neutrophil (NE-SFL) parameter in patients with APML against other hematological neoplasm's groups. On PCA, APML showed exceptionally significant variance for PLT, IPF, and NE-SFL. Through training of ANN predictive modeling, our selected set of attributes successfully classifies the APML group from non-APML groups at highly significant (0.894) AUC value with lower (2.3%) false prediction rate. Practical results of using our ANN model were found acceptable with value of 95.7% and 97.7% for training and testing datasets respectively which indicates that our set of parameters can represent a relevant "fingerprint" of a APML.

Conclusion: This approach expands the application of AI tools/models in clinical practice and indicate the results of basic blood test like CBC contain more information then clinician generally recognize. This CBC data driven AI modeling is a novel approach that substantially strengthen the predictive potential of CBC items, allowing the clinicians to be confident by the typical trend raised by these studied parameters and could initiate new prospects for clinical diagnosis

B-096

Soluble P-Selectin Levels in Patients with Sickle Cell Disease Reflect Platelets' Activation Rather than Endothelial Dysfunction

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Background: Mediators of adhesion have become a potential new target for pharmacological therapy to combat the complications of SCD. One of the molecules involved in this process is P-Selectin or CD62P, a cell adherence molecule that is rapidly and chronically expressed on the surface of endothelial cells and platelets when activated. Circulating soluble P-Selectin (sP-Selectin) appears to be slightly smaller than native P-Selectin. An alternatively spliced mRNA encoding a form of human P-Selectin lacking the transmembrane anchoring domain has been reported for both megakaryocytes and endothelial cells, and evidence suggests that the majority of circulating sP-Selectin arises in this manner. Recently, a humanized monoclonal antibody that binds to P-Selectin and blocks its interaction with P-selectin glycoprotein ligand 1 (PSGL-1) has been administered to patients with SCD, resulting in an amelioration of painful VOC. In this context we aimed to explore if sP-Selectin levels could be used to choose among the SCD patients those who might benefit from the new therapy. **Patients and Methods**: Eighty adult Caucasian patients with HbS/βthal at steady phase [40 patients under hydroxyurea (HU+) treatment and 40 patients without hydroxyurea (HU-) treatment] were included in this study, while 20 apparently healthy individuals of similar age and gender served as controls. Along with sP-Selectin levels, measured with the same method as reported previously (Human sP-Selectin/CD62P Immunoassay, R&D Systems, Minneapolis, MN, USA), other parameters of hemolysis, inflammation, endothelial dysfunction, iron accumulation and clinical features of the disease were evaluated. Results are expressed as median values ± SEM. **Results**: We found that sP-Selectin levels were elevated in 45/80 (56%) patients with HbS/βthal compared to controls (108.2±6.3 vs. 69.3±4.1ng/mL, respectively, $p<0.001$), independently of patients' βthal genotype and correlated strongly with PLT count ($r=0.760$, $p<0.001$). Regarding HU treatment, sP-Selectin levels did not differ between (HU+) and (HU-) patients (112.5±9.8 vs. 100.3±7.4ng/mL, respectively, $p>0.07$). No significance correlation was found between sP-Selectin levels and markers of: hemolysis (RPI: $r=0.191$, $p>0.100$); LDH: $r=0.103$, $p>0.360$ and bilirubin: $r=0.171$, $p>0.130$); inflammation (hs-CRP: $r= 0.002$, $p>0.842$); endothelial dysfunction (vWF:antigen: $r=0.141$, $p>0.210$ and ADAMTS-13: $r=0.089$, $p>0.507$). Regarding iron accumulation no correlation was found between sP-Selectin and ferritin levels ($r=0.090$, $p>0.438$), while a weak negative correlation was found with hepcidin-25 levels ($r=-0.283$, $p=0.018$). Furthermore, no correlation was found between sP-Selectin levels and history of clinical complications such as VOC ($p>0.795$), acute chest syndrome, venous and arterial thrombosis and mean pulmonary artery pressure values, ($p>0.402$). **Conclusion**: In this study, we proceeded with an external validation procedure of sP-Selectin determination in patients with HbS/βthal (keeping the same methodology in a different cohort of patients), and we found elevated levels of sP-Selectin with the evidence of PLTs' secretion origin, as no correlation found with other markers of endothelial dysfunction and inflammation. Interestingly, we failed to find a significant link of sP-Selectin levels with other markers of disease severity and/or clinical features of SCD. Thus, we consider that the use sP-Selectin as a biomarker of assessment and treatment of endothelial dysfunction in patients with SCD is of almost negligible importance.

B-097

Impairment of Glutathione System and Lipid Peroxidation in Patients with Hb H Disease: Possible Role of Hb H Concentration

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Background: Patients with HbH disease produce hemoglobin composed of a β_4-chains tetramer and have moderate to severe hemolytic anemia, a variable degree of ineffective erythropoiesis and splenomegaly. HbH is functionally characterized by high oxygen affinity, lack of subunit interaction, marked degree of instability and autoxidation. These result in the formation of insoluble particles within the RBCs which cause local oxidative damage, membrane dysfunction and shortened red cell survival. Glutathione (GSH) and the enzymes included by glutathione redox cycle are physiologic constituents of the intracellular antioxidant defense system and are present in almost all human cells. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA, thus MDA level is commonly known as a marker of oxidative stress. In this context we aimed to evaluate the RBCs' glutathione system and membrane lipid peroxidation along with antioxidant defense in patients with HbH disease. **Patients and Methods**: Twenty-seven treatment-naïve patients with HbH disease were included in the study, while 15 apparently healthy individuals served as controls. Along with hematologic and blood chemistry parameters and accurate determination of HbH levels, specific antioxidants (vitamins A, E and C) and catabolic products were measured as follows: red cell total glutathione (GSH_t), oxidized/reduced glutathione (GSSG and GSH_r respectively) levels by RP-HPLC with fluorimetric detection (excitation-385nm and emission-515nm); MDA levels by RP-HPLC with fluorimetric detection (excitation-532nm and emission-550nm); vitamins A and E levels by RP-HPLC with UV detection at 295nm, while vitamin C levels were measured by RP-HPLC with UV detection at 254nm. **Results**: Total glutathione as well as GSSG and GSH_r levels were significantly lower (by 25%, 45% and 17%, respectively) in patients with HbH disease compared to controls ($p<0.001$, $p<0.001$ and $p=0.02$, respectively). MDA levels were significantly increased (by 67%) in patients with HbH disease compared to controls. No significant differences were found in vitamin A and C levels between patients with HbH disease and controls ($p>0.341$ and $p>0.420$, respectively). Vitamin E levels were significantly reduced (by 50%) in

patients with HbH disease compared to controls ($p<0.001$), and correlate significantly with total bilirubin levels ($r=-0.458$, $p=0.03$). Ferritin and HbH levels correlated significantly only with MDA levels in patients with HbH disease ($r=0.423$, $p=0.03$ and $r=0.565$, $p=0.002$, respectively). **Discussion**: These results demonstrate two important issues: Firstly, the partial deficiency of red cell glutathione system points to increased oxidative status, due to HbH instability, denaturation and precipitation in the RBCs of the patients. The same is observed in patients with Sickle Cell Disease and might probably be due to altered concentrations of the metabolites serving as substrates in the glutathione system (e.g. L-glutamine). Secondly, the overall membranes' lipid peroxidation and manonyldialdehyde formation, which are mainly due to red cell HbH concentration and whole body iron accumulation, are detrimental to cellular integrity and function, which are further deteriorated by the partial deficiency of tocopherols. Thus, possible administration of glutathione system precursors and/or vitamin E could have beneficial effect in patients with HbH disease.

B-098

Performance Evaluation of the INNOVANCE Free PS Ag Assay on the Sysmex CS-5100, Sysmex CS-2500, and BCS XP Systems

M. Morowski. *Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany*

Background: Free protein S is a plasma protein that negatively regulates the coagulation pathway. If free protein S levels are reduced, patients show a significantly higher risk for venous thrombosis. A precise in vitro diagnostic test for free protein S antigen is important in the prophylaxis and therapy of patients experiencing such thrombotic events.

Aims: The INNOVANCE® Free PS Ag Assay is an immunoturbidimetric assay for quantitative determination of free protein S antigen in human citrated plasma. The aim of the studies was to evaluate the performance of the INNOVANCE Free PS Ag Assay on the Sysmex® CS-5100, Sysmex CS-2500, and BCS® XP Systems.

Methods: Assay precision was evaluated with three sample pools and two controls in a 20 x 2 x 2 (days, runs, replicates) design according to the CLSI document Evaluation of Precision of Quantitative Measurement Procedures - Third Edition. Method comparison studies of the INNOVANCE Free PS Ag Assay versus the STA-LIATEST Free Protein S assay using 350 patient samples were conducted according to the CLSI document Measurement Procedure Comparison and Bias Estimation Using Patient Samples - Third Edition.

Results: The INNOVANCE Free PS Ag Assay demonstrated high within-run and total within-instrument precision. Passing-Bablok regression results revealed a high correlation between results obtained with the INNOVANCE Free PS Ag Assay on the Sysmex CS-5100 System versus the STA-LIATEST Free Protein S assay on the STA-R EVOLUTION system. Good correlation was demonstrated between the INNOVANCE Free PS Ag Assay on the Sysmex CS-5100 System versus the same assay on the Sysmex CS-2500 and BCS XP Systems.

Conclusion: The INNOVANCE Free PS Ag assay shows good precision, compares well with the STA-LIATEST Free Protein S assay, and can be used for the quantitative determination of free protein S antigen in human plasma.

B-102

Interference of the Direct Factor Xa Inhibitors Rivaroxaban and Apixaban in the Determination of FVIII using Dade Actin FS and Dade Actin FSL Reagents

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Background: Rivaroxaban and apixaban are direct factor Xa inhibitors widely used for the prevention and treatment of thromboembolic diseases. However, due to their selective inhibition of factor Xa, which is an essential coagulation factor in the intrinsic coagulation pathway, rivaroxaban and apixaban are known to interfere with aPTT testing. **Aims:** Dade® Actin® FS and Dade Actin FSL reagents can be used in aPTT-based one-stage clotting assays for the determination of coagulation factor VIII in human plasma. The aim of the study was to determine at what concentrations of rivaroxaban and apixaban the interference with the determination of coagulation factor VIII using Dade Actin FS and Dade Actin FSL reagents becomes clinically relevant (relative deviation ≥10% [% of norm] factor VIII).

Methods: Interference testing was performed with plasma pools at 5, 40, and 70% of norm factor VIII using the paired-difference method and the dose-response method according to CLSI document EP07, Interference Testing in Clinical Chemistry - Third Edition.

Results: Paired-difference testing confirmed an interference of rivaroxaban and apixaban with the determination of factor VIII using Dade Actin FS and Dade Actin FSL reagents in 1:20 sample dilution on Sysmex® CS-2500 System. This interference leads to erroneously low results. Dose-response testing demonstrated interferences with rivaroxaban at concentrations >34.1 ng/mL and with apixaban at concentrations >86.9 ng/mL when determining factor VIII using Dade Actin FS and Dade Actin FSL reagents. **Conclusion:** Rivaroxaban and apixaban interfere with the determination of factor VIII using Dade Actin FS and Dade Actin FSL reagents. Rivaroxaban interferes at a lower concentration than apixaban. The interference concentrations can be reached by taking therapeutic doses of rivaroxaban and apixaban.

The poster presents exemplary data on interference testing with rivaroxaban and apixaban. These kinds of interferences are more or less pronounced by different aPTT reagents and present a general effect of direct oral anticoagulants on coagulation testing.

Disclaimer: Product availability may vary from country to country and is subject to varying regulatory requirements.

B-103

Diagnostic Management of Errors in Clinical Laboratory Test Selection

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Background: Diagnostic errors in clinical laboratory testing are extremely common and are major roadblocks in providing timely patient care. Clinical laboratory test menus have exploded in the past few years making it extremely difficult for clinicians to order appropriate tests. In 2017, Sarkar et al., Diagnosis, 4(1): 21-26, showed that about 78% of cases had an error in coagulation test selection evaluated by diagnostic management team (DMT) in real time, in an academic medical center with 450 beds. The purpose of this project was to investigate whether collaboration between the clinical laboratory, the DMT, and physicians who are ordering tests for a patient, resulted in improved test utilization and better patient care in the academic medical center. **Methods:** A retrospective study done between July,2017 - June,2019 evaluated whether improvement of test ordering was achieved that resulted in fewer errors in coagulation laboratory test selection for patients who presented with bleeding or thrombotic disorders. The study was undertaken after introduction of coagulation test panels and algorithms followed by test interventions by clinical laboratory, and real time evaluation of cases by the DMT. **Results:** A total of approximately 2400 coagulation test orders on patients were made between July, 2017 - July, 2018 and about 200 required intervention from the clinical laboratory. Between July, 2017 - Dec, 2017, 12% of cases resulted in intervention by clinical laboratory professional communicating directly with physicians and healthcare providers and educating them about new diagnostic algorithms and panels available for coagulation tests. The interventions dropped to about 5% for the period of next 6 months and was statistically significant at $p<0.05$. Furthermore, between July, 2018 - June, 2019, a retrospective analysis of coagulation cases that were evaluated by the coagulation DMT in real time, was undertaken to measure the impact of measures taken to standardize coagulation testing. A total of 634 cases of patients was evaluated by diagnostic experts in coagulation. The results revealed improvement of diagnostic errors in coagulation test selection and ordering by greater than 50% with error rate only at 36% compared to previously reported error rate of 78% in the Sarkar et al., study. The difference was statistically significant at $p<0.05$. All statistical calculations were made by using the N-1 Chi-squared test. **Conclusion:** This improvement by diagnostic management of errors in clinical laboratory test selection was achieved by effective collaboration between physicians, pathologists, residents and the clinical laboratory. A conservative estimate showed approximate savings of $10-12K annually, leading to improved patient outcomes and reduced cost burden. Hence, the dollars saved for very large academic medical centers can range between $80,000 - $120,000 annually. Thus the clinical laboratory and a DMT can function as an effective decision support system for test ordering, facilitate knowledge among care providers regarding test results and interpretation, that may help in proper evidence based guidelines and disease management.

B-106

Thrombus Formation through Upstream Activation-Downstream Adhesion of Platelets

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Background: Activation of platelets tends to cause adhesion and aggregation of platelets to the vascular wall. In circulatory environment, activation and adhesion may not occur at the same site since platelet activation requires time. Thus, we hypothesize that platelet activation at upstream and adhesion at downstream would be relevant in assessing the increased thrombotic risk associated with hyper-responsive vulnerable blood patients.

Methods: In this study, we designed a highly integrated microfluidic flow system to mimic the hemodynamic environment of vasculature with an upstream activation of platelets and downstream adhesion of platelets. Platelets can be activated by either agonists or shear stress generated with a rotational disk. Degree platelet adhesion was monitored by the migration distance (MD) of blood through the microchannel until it is blocked.

Results: Degree of pre-activation was widely varied with incubation time, agonist concentration and shear stress levels, which directly affected downstream adhesion of pre-activated platelets. There were linear relation between degree of activation and that of adhesion. However, excessive activation, rather, degraded downstream adhesion of platelets due to upstream aggregation and platelet lysis.

Conclusion: The results suggested that platelet adhesion was strongly dependent on the general conditions of upstream activation of platelets. This study implicates that downstream thrombus formation may be caused by upstream platelet activation rather than on-site activation.

B-109

A Long-Term Functional Outcome Analysis Suggested High AIS Patients with High NLR and PLR Recovery in Longer Time

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Background: Acute ischemic stroke (AIS) is a devastating disease and a leading cause of adult disability worldwide. The modulation of inflammation cell function plays a role in post-ischemic brain injury and repair. Increasing evidence suggest that systemic inflammatory response markers are , such as neutrophil to lymphocyte ratio(NLR), platelet to lymphocyte ratio(PLR), prognostic factors to functional outcome for AIS patients. In addition, the patients often need to receive long time recovery nursery. The aim of this study was to investigate the prognostic value of NLR and PLR upon ED admission for prediction of functional outcome over 12 months of AIS diagnostic. **Methods:** We retrospectively collected adult patients with AIS within 3 hours presented to the ED between 2016 and 2018, using a hospital stroke registry. The severity of AIS was assessed by the modified Rankin Scale (mRS) at ED admission, one month (M1), three months (M3), six months (M6), and one year (M12) after AIS. Neurological recovery was defined as an improvement of MRS≥1 scale compared to ED admission. **Results**A total of 277 patients were recruited in this study and blood parameters were collected from our emergency department prior to any treatment. We observed a general recovery rate of 38.6%, 27.4%, 27.7%, and 19.5% at 1, 3,6,12 months, respectively. In addition, we observed patients received rtPA recovery rate of 51.5%, 31.1%, 26.4%, and 21.6% ; received IA recovery rate of 32.1%, 9.0%, 28.3%, and 16.9%; received IA+rtPA recovery rate of 42.8%, 11.4%, 28.5%, and 22.8% at 1,3,6,12 months , respectively. The result of NLR and PLR were significantly decreased in the neurological recovery group compared with no improvement group at M1, but not at M3, M6, and M12 after AIS. An area under the receiver-operating characteristic curve (AUC) in predicting neurological improvement was 0.605 at M1, 0.581 at M3, 0.597 at M6, and 0.599 at M12 for NLR, and 0.572 at M1 for PLR. The patients with low NLR and PLR get a favoured function outcome. However, patients improved between 3 months to 12 months showed significantly higher NLR and PLR. Which indicate these patients requires longer recovery time. **Conclusion:**We have collected a large scale (277 patients) AIS functional outcome data for a long-term (12 months). The blood parameter PLR was firstly compared to the long term functional outcome data and suggested a patient with high NLR and PLR will be improved in long time.

B-110

Alinity hq PLT Counts: A Comparison with Two Platforms and Four Methods for Measuring PLT Concentrations

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Background: Technologies to determine platelet (PLT) count in whole blood include impedance, optical, fluorescence and immunolabeling methods by various automated hematology analyzers. The accuracy of PLT concentrations is especially important at medical decision levels, such as PLT transfusion thresholds. The Abbott Alinity hq hematology analyzer uses optical flow cytometry principles to determine PLT concentration in whole blood. PLT results obtained with this method were compared to those measured with a Sysmex XN-9000 hematology analyzer in the laboratory of Keio University (Tokyo, Japan), using the impedance (PLT-I), optical (PLT-O) and fluorescence (PLT-F) methods. In addition, PLT results were also generated on a subset of samples by dual-color flow cytometry (ICSH reference method). **Methods:** PLT concentrations were measured by Alinity hq and Sysmex XN-9000 on 348 patient samples. Additionally, 68 out of the 348 samples were tested with the ICSH reference method using a BD FACSCanto™ flow cytometer. Results were analyzed with Passing Bablok regression, Pearson correlation, and Bland Altman statistics. Predicted bias was calculated at medical decision levels. **Results:** PLT concentrations in the 348 samples were 6 - 957 x 10^9/L (by Alinity hq). PLT results produced by Alinity hq strongly correlated with all three Sysmex PLT methods (r=0.98 in each comparison). Using PLT-F as comparator, the mean differences were -4.0, -5,1 and -4.1 x 10^9/L for Alinity hq, Sysmex PLT-I and PLT-O values, respectively. In the 68 samples that were also assessed by flow cytometry, PLT counts spanned from 6.3 to 103.0 x 10^9/L. Results obtained with all four PLT methods demonstrated high level of correlation with the reference method on this cohort, with r=0.99 for Alinity hq and Sysmex PLT-O and PLT- F and r=0.96 for PLT-I. Mean differences ranged from -2.7 x 10^9/L (PLT-O) to 0.3 x 10^9/L (PLT-I). Predicted biases at the 10 and 20 x 10^9/L PLT transfusion thresholds were 0.0 and -0.5 x 10^9/L for Alinity hq, 0.3 and 0.2 x 10^9/L for PLT-I, 1.5 and 0.5 x 10^9/L for PLT-O and 0.0 and -0.4 x 10^9/L for PLT-F. **Conclusion:** All PLT methods correlated strongly with each other. Alinity hq PLT counts demonstrated high level of agreement with Sysmex PLT-F results. In addition, Alinity hq showed negligible bias compared to the ICSH-recommended flow cytometry reference method in the clinically relevant low PLT range and around PLT transfusion thresholds.

B-111

CALIPER Hematology Reference Standards on the Sysmex XN-3000 Analytical Platform: Improving Laboratory Test Interpretation in Children

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Background: Hematology laboratory tests are among the most routinely ordered in clinical care, with several important diagnostic and therapeutic implications. In pediatric hematology, accurate reference intervals (RIs) that reflect the dynamic physiological changes associated with growth and development are urgently needed for appropriate test interpretation. Unfortunately, critical gaps continue to exist in robust hematology RIs for modern laboratory platforms. To address this evidence gap, the current study establishes age- and sex-specific RIs for 25 hematology parameters on the Sysmex XN-3000 in the CALIPER cohort of healthy children and adolescents.

Methods: Fresh whole blood samples collected from a total of 566 healthy children and adolescents (birth to <21 years) with informed consent were analyzed for 25 hematological parameters on the Sysmex XN-3000 Hematology Analyzer. Age- and sex-specific reference standards were calculated based on the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Age- and sex-specific RIs were established for 25 hematological parameters, including erythrocyte, platelet, and leukocyte parameters. Of these 25 analytes, 16 required age-partitioning and five required sex-partitioning, highlighting the importance of covariate stratification in pediatric hematology test interpretation.

Conclusion: This study establishes a robust database of pediatric reference standards for hematological parameters in the CALIPER cohort on the widely-used Sysmex analytical platform for the first time. These data clearly demonstrate the dynamic and complex hematological profile in healthy children and can be expected to assist pediatric test interpretation in clinical laboratories that use the Sysmex platform.

B-113

Diagnostic Correlation between Microscopy and Flow Citometry in Chronic Lymphoproliferative Disease: An Experience of 758 Cases in One Center in Southern Brazil.

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Background: During one year we based our microscopic diagnosis of Chronic Lymphocytic Leukemia (CLL) in four different aspects as follows: Age above 50 years, persistent lymphocytosis (lymphocyte count ≥ 5.000 /mm3), presence of platelet clumping and/or macroplatelets and Gumprecht shadows in peripheral blood. The aim of the study was to evaluate our initial diagnosis of CLL with these parameters and compare with the final diagnosis by flow citometry.

Methods: In our laboratory we have a daily routine of 10 to 15 thousand samples of white blood cell counting. From this number we selected 768 patients with microscopic evaluation suggestive of CLL based on the aspects previously described. From this group, 253 (32,9%) were evaluated by flow cytometry test allowing us to compare the results obtained in both methods. As the other patients didn't have flow cytometry tests results available in our data, we maintained these patients as suspects of CLL based only on the microscopic analysis.

Results: According to the immunophenotypic score for diagnosis of Chronic Lymphocytic Leukemia (Matutes score), from the 253 patients, 201 (79,4%) had a score ≥ 4, indicative of CLL. The other 52 patients (20,6%) had a score ≤ 3 and received another diagnosis.

Conclusion: Flow cytometry studies should be done in all patients with complete blood cell count suggestive of Chronic Lymphocytic Leukemia because it can change the former diagnosis in at least 20% of patients.

Results obtained in flow cytometry of 253 patients with peripheral blood test suggestive of CLL	
CLL	201 (79,4%)
Normal / reactive	20 (7,9%)
Inconclusive	18 (7,1%)
Others (T cell, mantle, plasma cell)	14 (5,6%)
Total	253 (100%)

B-114

Sysmex XN-1000V™ Automated Hematology Analyzer Performance Comparison with Siemens ADVIA ® 120 in Dogs and Monkeys, Part I: Automated Complete Blood Count and Reticulocyte Parameter Comparisons

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BACKGROUND: Comparison studies between the Sysmex XN-1000V™ with multi-species software and the multi-species Siemens ADVIA® 120 hematology analyzers were performed assessing performance and suitability for drug discovery or nonclinical toxicological and efficacy studies. The Sysmex XN-1000V combines laser light, fluorescent stains, flow-cytometry and impedance, to perform complete blood counts (CBC), reticulocyte counts, and white blood cell (WBC) counts in whole blood. **OBJECTIVE:** The purpose of this study was to provide comparison between the Sysmex XN-1000V and the Siemens Advia 120 for analyzing whole blood from dogs and monkeys. Parameters measured and compared consisted of CBC including red cell indices, and reticulocyte counts. **METHODS:** Whole blood samples were collected approximately 6 months apart from two distinct cohorts of healthy, untreated, male and female Beagle dogs and Cynomolgus monkeys for correlation studies. Cohort 1 included 40 dogs and 40 monkeys and Cohort 2 consisted of 20 dogs and 20 monkeys. Sample analysis on both analyzers using multi-species software occurred within 4 hours of EDTA whole blood collection. CBC, total WBC and reticulocyte counts were captured. Separate analysis was performed for data from each collection. Data were combined for further analysis, increasing the N and allowing assessment of variation between cohorts. Platelet (PLT) counts for Cohort 2 were analyzed using both impedance and fluorescence on the Sysmex XN-1000V and compared to the Advia 120 light scattering methodology. EP Evaluator (Data Innovations LLC) was used to perform the comparison between analyzers producing correlation values, assessing bias and producing regression statistics for random and systematic error comparisons. **RESULTS:** All parameters, except MCH and MCHC, showed good to excellent correlation from individual cohort analysis for dogs (R≥0.84). Monkeys showed greater variability between cohorts. Cohort 1 monkeys showed very good to excellent correlation (R≥0.89) for all parameters except for MCHC (R=0.76). Cohort 2 monkeys showed more variability achieving excellent correlation for WBC, hemoglobin (HGB), and PLT (R≥0.98), good correlation for hematocrit (HCT) and mean cell volume (MCV) (R-values of 0.86 and 0.89 respectively), and fair correlation for red blood cells (RBCs) (R=0.77). Relative and absolute reticulocyte counts showed poor correlation (R=0.63-0.68). Cohort 2 showed no correlation for MCH and MCHC (R<0.50). When data from both cohorts were combined, good to excellent correlation was retained in dogs (R≥0.81) for all parameters except MCH, MCV, and MCHC. Combining monkey cohorts showed excellent correlation for WBC and HGB, good correlation for RBC, MCV, PLT, and both relative and absolute reticulocytes. Fair correlation was achieved for HCT and MCH. Both individual cohort analyses and combined analysis for both dogs and monkeys showed low bias (±10.0%) except Cohort 1 monkey PLT counts (bias=14.9%). PLT mode comparisons for both species exhibited very good to excellent correlation with low bias (-3.9 to 2.6%). **CONCLUSIONS:** The Sysmex XN-1000V performed comparably with the Siemens Advia 120 for CBC and reticulocyte counts in dogs and monkeys. While small differences were seen between cohorts, good to excellent correlation values and low biases indicate the Sysmex XN-1000V is acceptable for CBC and reticulocyte analysis in dogs and monkeys.

B-115

Sysmex XN-1000V™ Automated Hematology Analyzer Performance Comparison with Siemens ADVIA ® 120 in Dogs and Monkeys, Part II: *A*utomated and Manual Microscopic WBC Differential Count Comparisons

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BACKGROUND: Multi-species software for the Sysmex XN series of analyzers (XN-1000V) was released for use in research animals allowing comparisons with the Siemens ADVIA® 120 and manual microscopic methods. The Sysmex XN-1000V utilizes flow cytometry, fluorescent staining, and laser-based measurements of Side Fluorescent Light (SFL), Side Scatter Light (SSL), Forward Scatter (FSC) and Sysmex Adaptive Flagging Algorithm for Shape-recognition (SAFLAS) technology to characterize white blood cells (WBC) into a 5-part WBC differential. **OBJECTIVE:** The purpose of this study was to compare automated WBC differential counts between the Sysmex XN-1000V and Siemens Advia 120 analyzers in dogs and monkeys. Manual microscopic WBC differential counts were compared to automated counts from both analyzers to further evaluate performance. **METHODS:** Whole blood from healthy, untreated, male and female animals (60 Beagle dogs and 60 Cynomolgus monkeys) were analyzed side-by-side on Sysmex XN-1000V and Siemens ADVIA 120 automated hematology analyzers. Blood was collected at two timepoints approximately 6 months apart. Cohort 1 consisted of 40 dogs and 40 monkeys. Cohort 2 consisted of 20 dogs and 20 monkeys. Relative and absolute values were compared for the automated WBC differential counts for individual and combined cohort analyses. Two technologists identified and enumerated 200 leukocytes on separate blood smears from Cohort 1 animals. Averages of the two manual WBC differential counts were compared to values obtained from each instrument. Only relative values from the microscopic analysis were compared to instrument values. EP Evaluator (Data Innovations LLC) was used to perform method comparisons producing regression statistics including correlation coefficient (R), random error, and systematic error (absolute and percent bias) estimates. **RESULTS:** Comparisons of automated WBC differential counts from the Sysmex XN-1000V and Siemens Advia 120 hematology analyzers, showed excellent correlation (R≥0.95) for absolute and relative neutrophil, lymphocyte, and eosinophil values during combined and individual cohort analysis in dogs. Absolute monocyte counts showed fair correlation for the combined cohort analysis, poor correlation for cohort 1, and good correlation for cohort 2. Relative monocyte counts showed good correlation for cohort 2 but poor to no correlation for combined cohort and cohort 1, respectively. Basophil counts showed poor to no correlation. Good to excellent correlation (R≥0.84) for individual and combined cohort analysis was achieved in monkeys for both absolute and relative leukocyte counts except basophil counts. Manual WBC differential counts correlated well (R=0.81 to 0.88) to both analyzers for neutrophil and lymphocyte counts for dogs and monkeys. Monocyte and eosinophil counts showed poor (R=0.55 to 0.64) correlation, likely due to low numbers of cells available. Basophils were rarely seen on the blood smears resulting in no correlation. For WBC counts that correlated well, most parameters fall within ±25.0% bias with many showing less than 10.0% bias for both species. **CONCLUSIONS:** The Sysmex XN-1000V performed well when compared to the Siemens Advia 120 and manual microscopic WBC differential counts performed on dog and monkey whole blood. The relatively low bias and high correlation achieved indicate the Sysmex XN-1000V is suitable for use in nonclinical studies supporting drug development.

B-116

Evaluation of the Results of a Mass Transfusion Protocol

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Background: In situations of massive hemorrhage, the need for large amounts of blood products implies a significant consumption of resources and the involvement of several specialties. Due to the high morbidity and mortality in these cases, we developed a massive transfusion protocol (MTP) in December 2015, with the aim of achieving an efficient blood supply and using fixed transfusion components, which seems to reduce mortality by 74%.The objective of this study was to review and evaluate the results obtained from the massive transfusion protocol in 2019.

Methods: Evaluation of the data of patients who have suffered massive hemorrhages in our hospital during 2019, after MTP implantation. Database: Diraya Clinical Station and e-Delphyn 8.0.5.0. Analysis of the results through Microsoft Excel 2010.

Results: In the period of time studied there were 20 cases of massive transfusion (8 A+, 7 0+, 2 B+, 2 AB+ and 1 0-). The average age was 57 years (32-81), 14 were men (70%) and 6 women (30%).The summary causes of massive hemorrhage collected were: rupture of abdominal aortic aneurysm (4), digestive hemorrhages (4), complications in major intra-abdominal surgery (5), obstetric surgery (3), orthopedic surgery (1) and serious pancreatitis complications (3). There were 5 patients, 3 during the procedure and 2 later, due to complications secondary to their pathologies after a good response to the massive transfusion of blood products in the critical situation. A total of 175 red blood cell concentrates (RBC), 111 plasmas and 18 platelet pools were sent. As for the RBC, 4 were 0- bags reserved for cases of extreme urgency, 138 were isogroup to the patient and the remaining 33 were isocompatible. In none of the cases were incidents recorded throughout the procedure, inherent to the performance of the different specialists involved, to the quantity and time of delivery of blood products, or complications in patients due to post-transfusion reactions.

Conclusion: The non-existent notification of incidents during the MTP indicates that the protocol has presented a very favorable implantation and dissemination, both in the laboratory staff and in the hospital services involved (mostly ICU, Surgery and Anesthesia).The RBC stock of our laboratory is agreed with the reference transfusion center, based on the population proportion of blood group. Therefore, in three of the patients with massive blood products needs with more minority groups (B and AB), isocompatible concentrates were transfused, without incident. The overall mortality of patients requiring massive transfusion ranges between 30 and 69%, depending on the process that triggers it, and in our case we talk about 25%, a fact that confirms the good management of critical situations in these patients with the MTP. In some of these cases, the activation-deactivation of the protocol is not reflected, an aspect that could be reviewed for better operation and future evaluations.

B-117

Identification of Hereditary Disorders of Hemoglobin by HPLC

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Background: Hereditary hemoglobin (Hb) disorders are among the most common monogenic diseases and affect approximately 7% of the world's population. Every year 300,000 affected children are born around the world whose parents are healthy carriers, so neonatal screening is vital to provide families with genetic advice.These diseases can be divided mainly into structural hemoglobinopathies (anomalous Hb synthesis) and thalassemias (decreased Hb synthesis); there are also thalasmic hemoglobinopathies and hereditary persistence of fetal Hb (HPFH: synthesis of HbF during adulthood). There are more than 1800 known mutations in human Hb and new ones are still being discovered, although most have a very low prevalence.

The objective of this study was the identification of patients with inherited Hb disorders by analyzing the chromatograms obtained after the measurement of glycosylated hemoglobin (HbA1c).

Methods: Descriptive and retrospective study of all patients with inherited Hb disorders identified by glycosylated hemoglobin measurement. For this, all HbA1c chromatograms obtained by cation exchange HPLC (high performance liquid chromatography) on the Tosoh G8 analyzer from Horiba were analyzed. In total, more than 65,000 chromatograms performed during the year 2019 in our hospital were reviewed.

Results: 40 patients with inherited Hb disorders were identified, in addition to another 15 patients with HbF values greater than 5%. The most frequently identified Hb variants in our hospital were HbS and HbC, with 14 patients in each group. Of the patients with HbS, 5 were from Africa and 3 from South America, while from the patients with HbC, 4 came from Africa and another 3 from South America.

In addition, 3 patients with HbD were identified, 2 with Hb Valme and 2 with Hb Fukuyama (one of them with an associated alpha-thalassemia). We also identified 2 patients with HPFH, one with 10% HbF and another with 25% HbF. Finally, there are 3 other patients with Hb variants pending identification by molecular characterization. Most patients were previously diagnosed with these diseases and the Hb variant does not interfere with the measurement of HbA1c by HPLC. Only in patients with Hb Valme and Hb Fukuyama, this variant interferes with the measurement of Hb1Ac by HPLC and its analysis was necessary by the boronate affinity method.

Conclusion: The measurement of HbA1c by HPLC allows us to identify patients with inherited Hb disorders, being very important to know the probability of their children to suffer from this disease. The use of other methods of measuring HbA1c, such as the turbidimetric inhibition immunoassay (TINIA), does not allow the detection of these variants in patients.

HbS and HbC are more prevalent in patients from Africa and South America. This has been verified in our hospital, since 54% of these identified variants corresponded to patients from these regions.

B-118

Comparative Evaluation of the Alinity hq Hematology Analyzer on an Onco Hematology Patient Population

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BACKGROUNDThe Abbott Alinity hq is a fully automated hematology analyzer, which provides a 6-part WBC differential that includes immature granulocytes (IG). The analyzer reports nucleated red blood cell counts (NRBC) with every CBC+Differential test. Alinity hq uses an advanced version of the MAPPS™ (Multi Angle Polarized Scatter Separation) technology, built on optical and fluorescence principles. This study has focused on the analytical performance comparison between Aliniy hq and Sysmex XT 2000i on an onco-hematology patient population at Hammersmith Hospital (London, UK).

METHODTotal 230 samples were evaluated for method comparison with the Alinity hq and the Sysmex XT 2000i. Results were analyzed with Pearson correlation and Passing Bablok or Deming regression. Two independent readers performed a 100-cell WBC differential and the results were used to assess correlation and agreement between Alinity hq and manual WBC differential.

RESULTSHigh level of correlation and agreement have been demonstrated between the two analyzers for CBC and WBC differential parameters, with correlation coefficient (r) spanning between 0.82 and 0.99, except for %basophils (r=0.40). The default impedance platelet count (PLT-I) by XT 2000i showed strong correlation (r=0.98) when compared with the Alinity hq PLT count. A subset of samples (n=72) were reflexed to optical PLT count (PLT-O) on the Sysmex XT 2000i. These results also reflected good correlation (r=0.98), with slight positive bias by Alinity hq. Only 26 samples were available for % Reticulocyte comparison. The results showed strong correlation (r=0.98) and small positive bias by Alinity hq.Comparison of Alinity hq WBC differential to manual differential demonstrated excellent correlation for %neutrophils (r=0.96) and %eosinophils (r=0.93), moderate correlation for %lymphocytes (r=0.85) and %monocytes (r=0.72) and weak correlation for %basophils (r=0.37), with positive bias for %monocytes and negative bias for %neutrophils; a known finding in method comparisons between automated and manual WBC differential. %IG (promyelocytes, myelocytes and metamyelocytes) also demonstrated strong correlation (r=0.88) but appeared to be slightly lower by Alinity hq. NRBC correlation was weak (r=0.55), but there were only 4 positive samples agreed by both readers with >1 NR/W in the cohort.**CONCLUSION**Alinity hq demonstrated equivalent performance with the XT 2000i hematology analyzer. WBC differential results showed high level of accuracy compared to the manual differential.

B-119

The Laboratory Algorithm for Screening and Diagnosis of Hemoglobinopathies

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Background: Disorders of globin chain synthesis are among the most common genetic disorders worldwide. Russia was a country with a low prevalence of this pathology a long time. But now non endemic countries are also involved in this problem as a result of demographic changes caused by migration of ethnic groups with a high frequency of hemoglobinopathy. There is need to create an algorithm for screening and diagnosis of hemoglobin disorders, that includes available laboratory methods and technologies.Objective. To create and approbation a laboratory algorithm for the early diagnosis of hemoglobin disorders for low prevalence areas. **Methods.** The study was carried out in the North-Western region of Russia. The algorithm was created on the basis of an examination of 150 patients (from 1 to 58 years) with hemoglobin disorders. The comparison group consisted of 200 patients (from 1 to 54 years) with microcytic (MCV<80 fl) hypochromic (MCH<27 pg) anemia due to other cases (primarily, iron deficiency).The diagnosis of hemoglobinopathies involves measuring RBC parameters (Sysmex XN-2000, Japan) and calculated indices that incorporate at least 2 of the RBC parameters provided by the automated hematologic analyzers; quantification assay of hemoglobin fractions by capillary electrophoresis (MINICAP, Sebia, France). Molecular characterization of mutations is performed with reverse-hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines (ViennaLabDiagnostics, Austria).The samples were collected in EDTA anticoagulant tubes.**Results**. It was found that the most common form of hemoglobin disorders in the North-Western region of Russia is b-thal (92%). Other forms include a-thal, hemoglobinopathies S, D, C.The laboratory algorithm for the early diagnosis of hemoglobin disorders for low prevalence areas was created:

Step 1 - screening. It is based on RBC parameters and combined use of the index M (Menzer) = MCV/RBC and Si (Sirdah) = MCV - RBC - 3 × Hb with cutoff for detecting thalassemia <11.5 and <25, respectively (ROC-analysis: AUC 0.970, Sensitivity 87.5%, Specificity 97.5%, Youden Index 85%). It is an available method for identifying patients with a high probability of thalassemia in all laboratories with an automatic hematologic analyzer.In the case of rare form hemoglobin disorders screening is based on assessing the individual risk by determining the family origin of the patient by the questionnaire.Step 2 - the study of hemoglobin fractions for all patients with a high probability of hemoglobin disorders (from step 1). The capillary electrophoresis was an effective method that allowed to confirm the diagnosis in the study in 98% of cases (with the exception of a-thal, when changes of fractions are not detected).Step 3 - molecular analysis. The most frequent b-thal mutations in this population were codon 8 (-AA) - 36% and IVS 1.110 (G>A) - 26%. It is performed if genetic counseling is required or for patients with suspected a-thal.**Conclusions.** The algorithm (step 1+2) has a sensitivity and specificity of 98%. It is effective and accessible for areas of low prevalence and allows to directly search for carriers of hemoglobin disorders without additional costs.

B-120

Multi Platform Comparison of the Alinity hq Hematology Analyzer

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Background The performance of Abbott Alinity hq high throughput hematology analyzer was evaluated, in comparison with Siemens Advia 2120i and Beckman Coulter DXH 800 at laboratory of Medilab66 (INOVIE) (Saint-Laurent-de-la-Salanque, France). **Methods** The sample cohort consisted of 551 samples, mostly from outpatients. The samples were run on the Alinity hq analyzer after being tested with both the Siemens Advia 2120i and the Beckman Coulter DxH 800 instruments. In addition, a peripheral blood smear was made for each sample and a 2x100-cell WBC differential was performed by two experienced hematologists. Results were compared using Passing-Bablok or Deming regression, Pearson correlation and Band Altman statistics. **Results** In the Alinity hq vs Advia 2120i comparison strong correlation and agreement were obtained for all RBC parameters with correlation coefficients (r) ranging from 0.97-1.00 and regression slopes ranging from 0.96-1.04. High level of correlation and agreement was observed for WBC (r=1.00), %NEU (r=0.99) and %LYM (r=1.00). Moderate correlation was observed for %EOS (r=0.83) and weak correlation for %BASO (r=0.37). Despite the strong correlation for %MONO (r=0.90), the slope of 1.47 suggested a significant bias between Alinity hq and Advia 2120i results. Manual WBC diff confirmed Alinity hq %MONO results, implying that Advia 2120i has underestimated this measurand. Strong correlation (r=0.99) and agreement was observed between Alinity hq and Advia 2120i platelet (PLT) counts across the AMR, but correlation was weak for MPV (r=0.33). To focus on the clinically important low PLT counts, a separate comparison was performed on a subset of samples with PLT counts of < 200 x 10^9/L (n=160), and the results demonstrated good agreement between the two instruments with a slope of 1.06 and an r value of 0.97.The Alinity hq vs DxH 800 comparison demonstrated close correlation and agreement for most measurands (r=0.93-1.00), except for %BASO (r=0.57). Bland Altman analysis showed a mean bias of -1.96% for RDW-CV despite excellent overall agreement for MCV results. Alinity hq results closely matched the manual %NEU and %LYM results; moderate correlation was observed for %MONO and %EOS, and weak correlation for %BASO (r=0.48). Bland-Altman statistics showed a small negative bias for %NEU (-3.2%), and a small positive bias for %LYM (2.0%) and %MONO (1.6%). Good correlation was also observed for immature granulocyte percentage (%IG) and nucleated red blood cells (NR/W) (r=0.88 and 0.91, respectively), with -0.5% mean bias between Alinity hq and manual %IG results.**Conclusion** Good overall agreement was demonstrated between results generated by the Abbott Alinity hq, the Siemens Advia 2120i and the Beckman Coulter DxH 800 hematology analyzers. The observed differences are consistent with the technological differences, such as Advia 2120i reporting large unstained cells (LUC) in the WBC differential, and DxH 800 using impedance technology to obtain the RBC volume (and consequently, RDW), whereas Alinity hq using optical light scatter principle. Alinity hq provides accurate complete blood count and WBC differential results, including IG and nucleated red blood cell concentrations.

B-122

Reducing False-Positive Platelet Clump Flags on the Sysmex XN 9000 Analyzer

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Background: When platelets stick to each other, they form clusters or clumps. This phenomenon will cause automated hematology analyzers such as the Sysmex XN9000 to spuriously report a lower platelet count. However, the XN9000 generates a flag (PLT Clumps) to alert laboratory technologists that platelet clumps are present. We then prepared a blood smear from the blood sample, review the slide and verify the presence of platelet clumping. The XN9000 analyzer has three different methods for the counting of platelets: Impedance (PLT-I), Optical (PLT-O) and fluorescence (PLT-F). We currently use PLT-O to avoid counting cellular elements of similar size (microcytes and fragments) as platelets when impedance is used. Recently, we encountered an increase in the number of false positive PLT Clump flags with the XN9000 necessitating more slide reviews. **Methods:** The objective of this study was to investigate the utility of the PLT-F mode on the XN9000 analyzer in the reporting of the PLT Clumps flag. The PLT-F on the XN9000 uses a platelet-specific fluorescent dye, oxazine, to stain platelet mitochondria and surface endoplasmic reticulum. The study was conducted over a month. All samples that had the PLT Clump flag on the XN9000 when analyzed on the PLT-O mode were reflexly reanalyzed on the PLT-F mode. A slide review was also performed on all these samples to confirm the presence or absence of platelet clumps. This derivation cohort was then compared to a validation cohort analyzed on the PLT-F mode for a month. A slide review was also performed on all PLT Clump-flagged samples to assess the presence of platelet clumps. **Results:** In this derivation cohort of 17205 samples, 428 samples (2.5%) exhibited the PLT Clump flag with 39% of them reporting a platelet count below 150,000/uL. Slide review of these 428 samples showed that only 174 samples were positive for platelet clumps (40.7% true positives) while 254 samples had no platelet clumps (59.3% false positives). With the PLT-F method, 173 of the 174 samples with platelet clumps were correctly tagged with the PLT Clump flag (99.4%) while the 254 samples without platelet clumps were correctly identified (100%). In the validation cohort (n=17418) using PLT-F for 1 month, only 228 samples (1.3%) bore the PLT Clump flag - a 48% relative reduction in slide review. **Conclusion:** The PLT-F method accurately classifies platelet clumps. We now employ this strategy - primary blood cell count results with the PLT Clump flags are auto-reflex to be reanalyzed on the PLT-F mode with slide review performed if the PLT Clump flag persists. This new workflow has substantially decreased our slide review rate for the PLT-Clump flags.

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Comparison of Alinity hq with Sysmex XN 3000 suggests Equivalent Analytical Performance

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Introduction Fully automated hematology analyzers utilize a range of technologies, including /impedance, conductivity, optical and fluorescence flow cytometry . Due to methodological differences and their unique characteristics, it is important to correlate results below, within and above reference intervals when evaluating new technologies, as per CLSI recommendations. This study focused on comparing complete blood count (CBC), WBC differential and reticulocyte results between the Abbott Alinity hq hematology analyzer and the Sysmex XN-3000. Alinity hq uses an advanced version of Abbott's patented Multi Angle Polarized Scatter Separation (MAPSS™) technology in a single channel approach. Sysmex XN-3000 employs light scatter and fluorescence along with impedance in a multichannel approach. **Methods** 467 samples were selected for method comparison in the laboratory of San Giovanni Calibita Fatebenefratelli Hospital, a multispecialty health care center in Rome, Italy. The cohort included samples from patients with several types of anemia and leukemia along with normal specimens. Results were analyzed with Pearson correlation and Passing Bablok or Deming regression. **Results** High level of correlation and agreement was demonstrated between the two analyzers for WBC and differential parameters, including %immature granulocytes (%IG) as well as nucleated red blood cells (NRBC). Close correlation and agreement was also obtained for all RBC parameters as demonstrated by the slope, intercept and correlation coefficient values in Table 1. Alinity hq default optical PLT counts were compared with Sysmex PLT counts obtained with the PLT-F channel of the XN-3000. These results also demonstrated excellent correlation in all samples and in a subset of samples with PLT ≤ 100 x 10^9/L. Data showed negative bias for MPV, possibly reflecting technological and methodological differences.

Conclusion The advanced MAPSS™ technology in a single channel approach by the Alinity hq analyzer demonstrated comparable performance with the Sysmex XN-3000 technologies in a high-volume laboratory.

Table 1. Alinity hq vs Sysmex XN-3000 Method Comparison Results

Measurand	N	Range tested (Alinity hq)		Passing-Bablok		Pearson's r
		Min	Max	Slope	Intercept	
RBC (x 10^{12}/L)	452	1.32	6.65	0.99	0.06	0.98
MCV (fL)	455	54.20	127	1.06	-5.88	0.98
RDW-CV (%)	460	10.70	30.20	0.91	0.00	0.89
MCH (pg)	452	16.90	42.04	0.93	2.08	0.99
HCT%	452	15.20	51.40	1.05	-2.02	0.98
HGB (g/dL)	453	5.07	17.20	1.00	0.20	0.99
%RETIC	452	0.00	12.90	1.17	0.08	0.96
PLT (x 10^9/L)*	445	6.50	948	1.02	0.73	0.99
PLT (x 10^9/L)**	24	6.50	128	1.06	0.48	0.96
MPV (fL)	410	5.11	10.80	1.25	-5.50	0.81
WBC (x 10^9/L)	464	0.73	314	1.00	0.00	0.99
%NEU	414	29.60	93.80	0.97	1.10	0.99
%LYM	383	1.99	62.10	1.00	0.03	0.98
%MONO	413	1.44	36.60	1.02	-0.22	0.95
%EOS	436	0.01	17.60	1.00	0.00	0.98
%BASO	447	0.00	3.33	1.38	-0.23	0.66
%IG***	414	0.00	8.38	0.62	-0.10	0.72
NR/W****	463	0.00	32.70	1.00	-0.03	0.97
* PLT-F on Sysmex, all samples						
**PLT-F on Sysmex, PLT ≤100 x 10^9/L						
***Deming Regression						
****Deming Regression, Nucleated RBCs per 100 WBC						

B-129

Validation Study of ADAMTS13 Activity Assay

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Background: Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease that presents clinically with microangiopathic hemolytic anemia, thrombocytopenia, and microvascular blood clots. It is important that TTP is differentiated from hemolytic uremic syndrome for effective treatment by plasma exchange. ADAMTS13 is a circulating metalloprotease produced by hepatic stellate cells and vascular endothelial cells that cleaves ultra-large von Willebrand Factor multimers. Decreased plasma ADAMTS13 activity has been shown (<10%) in congenital and acquired TTP. ADAMTS13 activity assay is a specific laboratory test for the diagnosis of TTP. Because our laboratory currently sends this test to a reference laboratory, physicians may have to make clinical decisions while waiting for test results. Recently, our hospital decided to bring this test in-house. We report here the validation study of a plasma ADAMTS13 activity assay on an automated system.

Methods: The ADAMTS13 activity assay is an enzyme-linked immunosorbent assay (Technoclone, Vienna, Austria). ELISA test strips are coated with monoclonal anti-GST antibody. The substrate is a GST linked vWF73 peptide with the cleavage site Y1605-M1606. The detection antibody horseradish peroxidase (HRP) conjugated monoclonal anti-N10 antibody binds to C-terminal residues D1596-Y1605 after cleavage. The HRP substrate tetramethylbenzidine's diimine product is quantified at 450 nm wavelength. The ADAMTS13 activity assay is performed an automated system. All calibrator, control and specimens were run in duplicate to ensure precision. Assay precision, analytical measurement range, limit of quantification, linearity, sample stabilities, and interference studies were carried out according to CLSI and institutional guidelines.

Results: The ADAMTS13 enzyme activity is usually expressed as IU/mL or percentage of normality (1 IU/mL = 100%). The precision study was carried out using two levels of pooled QC specimens run in duplicate. The interday CV and intraday CV for both specimens were within the expected range of 15%. To compare assay performance in two Quanta-Lyzer instruments, 13 specimens over a range of 0.14 - 0.86 IU/mL were analyzed on the two systems. Deming regression analysis showed slope and intercept values were 0.92 (95% CI: 0.79-1.04) and 0.06 (95% CI: -0.02-0.13), respectively, with a correlation coefficient (R) of 0.98. The linear dynamic range was 0.05-0.8 IU/mL. Twenty-five specimens were sent to a reference laboratory for method comparison studies. The results showed good correlation with correlation efficiency of 0.93 and Deming regression slope of 0.90 (95% CI: 0.75-1.05). The presence of bilirubin, hemoglobin, or triglycerides did not interfere with the assay. The accuracy of this method was evaluated using CAP proficiency test specimens. The manufacturer established the normal range as 0.4-1.3 IU/mL. We verified the reference range for the assay by running 25 specimens from apparently healthy donors. ADAMTS13 activity below 0.4 IU/mL we considered abnormal.

Conclusion: We implemented an ELISA-based ADAMTS-13 activity assay on an automated ELISA platform. The in-house reference interval study verified the manufacture established reference range. The critical value for ADAMTS-13 activity is <0.40 IU/mL. Bringing ADAMTS13 activity assay in-house will greatly improve the test turnaround time and provide a valuable tool for the diagnosis and treatment of patients with TTP.

B-130

Investigation of Blood Stabilizers as Alternative QC for Hematology Analyzers

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Background: Quality control material is produced for automated hematology analyzers primarily by one manufacturer in the United States. Prolonged supply chain interruptions would compromise the ability to perform QC analysis. The objective of this study was to determine the suitability of using laboratory developed hematology QC materials for the Sysmex XN automated hematology analyzer. Methods: Suitability of two blood stabilizers was evaluated. First, TransFix™ (Cytomark, UK) was added per manufacturer instructions to 10 whole blood samples collected into EDTA and a CBC with differential were performed once every 24 hours for 18 days. Second, 1 mL of laboratory prepared stabilizer (37% formaldehyde, 50% glutaraldehyde, 99% tri-sodium citrate in distilled water) was added to 49 mL of sample prepared by combining a leukoreduced RBC unit and PLT unit. A CBC was performed once every 24 hours for 5 days, stored at 4℃ for 30 days then tested once every 24 hours for 28 days. Evaluation of both stabilizers as alternative QC for the Sysmex XN by comparing coefficient of variation (CV) guidelines from Clinical and Laboratory Standards Institute (CLSI) and XN-Check™ QC (Sysmex, USA, lot 81221101) CVs, excluding white cell parameter for the laboratory prepared stabilizer. Results: See Table 1. TransFix CVs were below CLSI CVs guidelines except for MCV and MPV. XN-Check QC CVs for TransFix samples were acceptable for all parameters except MONO%, RDW, EOS%, and BASO% (data not shown). The laboratory-developed stabilized sample CVs were below or within CLSI and XN-Check limits for all CBC parameters except MPV by CLSI. Conclusions:TransFix and the laboratory prepared stabilizers were

suitable as alternative QC on the Sysmex XN hematology analyzer for most hematology parameters. Laboratories should consider these alternatives when commercial QC materials are not available.

Table 1. Result Comparison of Alternative Hematology QC Parameters to CLSI and XN-Check QC CVs

Sample Number	RBC (%CV)	HGB (%CV)	HCT (%CV)	MCV (%CV)	PLT (%CV)	MPV (%CV)	WBC (%CV)	NEUT% (%CV)	LYMPH% (%CV)
CLSI	3.2	2.8	2.8	1.3	9.1	4.3	10.9		
XN-Check QC	3.9-4.8	3.9-4.9	5.9-6.7	5.9-6.0	8.9-38.3	5.4-16.0	6.0-10.0	10.0-14.1	21.9-25.1
QC-TF001 n=18	0.8	1.3	1.6	1.4	8.3	3.0	2.9	3.7	22.1
QC-TF002 n=18	1.6	0.6	2.1	2.3	5.6	1.0	2.1	4.1	8.5
QC-TF003 n=18	0.9	0.8	1.0	0.7	7.0	4.8	2.4	5.1	18.6
QC-TF004 n=18	0.6	1.0	0.9	0.8	5.3	3.4	2.4	2.6	10.7
QC-TF005 n=18	0.7	1.0	0.9	1.1	6.3	4.4	2.5	8.6	13.7
QC-TF021 n=18	0.8	0.6	2.6	2.9	3.8	3.4	2.1	3.3	14.0
QC-TF022 n=18	1.3	1.3	2.0	1.7	3.3	3.2	3.0	4.9	17.8
QC-TF023 n=18	0.8	1.6	2.3	1.9	2.3	2.7	1.6	6.3	13.5
QC-TF024 n=18	0.8	1.0	2.1	2.2	3.5	2.8	2.0	2.3	9.0
QC-TF025 n=18	0.8	0.9	2.2	1.9	2.9	2.3	1.6	4.1	6.4
QC-LD n=29	0.7	0.6	1.1	0.8	3.2	5.9	*	*	*

TF = TransFix
LD = lab-developed
Above CLSI %CV
* QC-LD leukodeduced no WBC parameters tested

B-131

Evaluation of New Automated Hematopoietic Progenitor Cells Analysis Performance in the Peripheral Blood Stem Cell Apheresis

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Background: Peripheral blood stem cell transplantation (PBSCT) is suitable for diseases such as hematological malignancies, bone marrow or stem cell defect, and germ cell cancer. In the past, the peripheral blood white cell count(WBC) has been used as a marker to stem cell mobilization by clinicians. Previous studies indicated that the correlation between peripheral blood hematopoietic progenitor cells (HPC) derived from some automated analyzers and CD34 + stem cells is better than peripheral blood WBC as the marker to predicit it . This study evaluated the new automated HPC count on Sysmex XN-9000 hematology analyzer in PBSCT.

Methods: In this study, we were collected 41 patients undergoing an autologous transplantation , including 27 males and 14 females from September 2018 to December 2019. Peripheral blood and apheresis samples (n=65) were anticoagulated with K_2EDTA. WBC and HPC counts in peripheral blood and apheresis samples were examined using Sysmex XN-9000 hematology analyzer. CD34 + stem cells in apheresis samples were examined using BD Canto II flow cytometery analyzer on the same day.

Results: The results showed that preharvest peripheral blood HPC was related to CD34 + stem cells (r = 0.90) ; apheresis HPC was related to CD34 + stem cells (r = 0.95), and both performed good correlation. The correlation between peripheral blood WBC count and CD34 analysis was poor (r = 0.12). And if the collection amount reaches 2 x 10^6 CD34 + cells / Kg as the criterion for predict successful stem cell collection, it is calculated that when the PB XN-HPC cut-off value is 22.5 cells / µL , ROC Curve AUC = 0.935, and Sensitivity is 95.7%, Specificity is 81.0%, Positive predictive values is 73%, Negative predictive values is 97%, and the consensus rate is 86%.

Conclusion: Recent study demonstrated HPC analysis by Sysmex XE series hematology analyzer with CD34+ stem cell showed only moderate correlation (r = 0.50). This study showed that the new automated hematology Sysmex XN-9000 analyzer performed better on the correlation between PB-HPC and PBSC CD34 + stem cells (r = 0.90). Although the use of PB specimens to measure CD34 + stem cell content in clinical practice can more accurately and effectively predict the effectiveness of stem cell collection, it is limited by the size of each hospital's laboratory , the labor and cost of performing CD34 + stem cell tests. The XN-9000 hematology analyzer can also be used to quickly and cost-effectively predict the time point of stem cell collection with HPC and cut-off values.

B-132

Hematology QC Survey Indicates that WBC, Neutrophils, Platelets, Lymphocytes and Hemoglobin are Minimally 3 Sigma and that Hemoglobin should be Reported Instead of Hematocrit

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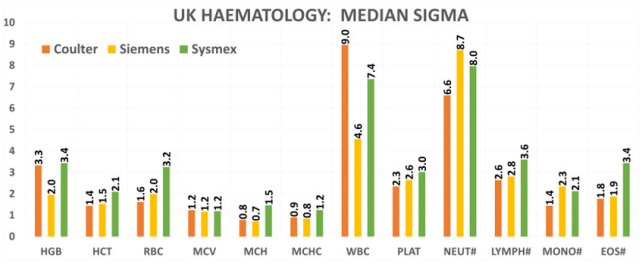
Background: Under the auspices of the International Committee for Standardization in Hematology (ICSH) we conducted a survey of QC practices in automated haematology. The ultimate purpose of this survey was to provide guidance for selecting optimal QC rules for each of the complete blood count (CBC) components. Laboratories provided estimates of their long term normal level QC averages and standard deviations for each CBC component. Sigma, a measure of analytical goodness was derived from the ratio of Biologic Variation (BV) to Analytical Variation (AV) and helps select optimal QC rules. Based on the BV and AV, we simulated patient results to demonstrate the effect of sigma on specific components. **Methods:** Survey participants recorded instrument model, laboratory environment, QC frequency, usual QC range and mean and standard deviation of the normal level QC at 60 days. Sigma was derived for each laboratory and CBC constituent from published BV estimates and survey-determined QC AV. We simulated the analysis of both low and high sigma analytes. As clinicians primarily classify normal and abnormal based on institutional reference intervals, we simulated serial patient data close to their reference limits. **Results:** 55 survey results represented 3 manufacturers: Coulter:(1 DXH and 7 DXH 800); Siemens:(14 Advia 2120); and Sysmex:(9 XE2100 and 24 XN). The Figure shows median QC defined sigmas. **Conclusion:** Use of tests with low sigma (< 2) will result in analytical error affecting the accuracy of the measurement. Reporting of Hgb rather than Hct will always provide a more useful, less variable measure. Luckily, the actionable tests, those which are associated with specific clinical protocols, generally have sigmas exceeding 3, and thus produce highly reproducible results.



B-133

Comprehensive Process to Evaluate High Range Hematology Analyzers with Robust Indicators like Efficiency, Slide Review Rate, Flags Sensitivity and Flags Specificity

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Background: Understanding of efficiency, slide review rate, sensitivity andspecificity of flags of high range hematology analyzers is important for large-mid size laboratory for their flawless workflow. Various high-end hematology analyzers are available in the market and claim about their best solution for the laboratory. This study gives the real time effectiveness and efficiency of High-end hematology automation on following three parameters 1. Efficiency, 2. Slide review rate, 3. Flags sensitivity, 4. Flags specificity

Methods: Four high-end automated hematology analyzers Yumizen H2500 (HORIBA Medical, France), DxH800 and DxH900 (Beckman Coulter Inc, USA), XN9011 (XN10) (Sysmex corporation, Japan) have been studied on above-mentioned four parameters. Patient samples were ran on various analyzers after the qualified installation conditions of manufacturers. We created two sets of 200 samples totaling 400 samples of this study. We processed one set of 200 samples on Yumizen H2500, DxH800 and DxH900 while second set of 200 samples was processed on Yumizen H2500 and XN 9011(XN10). We analyzed the claim of throughput and performance of throughput in working laboratory to measure the efficiency. We measured slide review rate, flags

sensitivity and specificity after preparing the slides of all non-validated samples due to flags generated by the analyzers. We digitalized slides on DM1200 (CellaVision, Sweden) analyzer. We verified slides manually as well

Results:we measured efficiency in laboratory of above mentioned hematology analyzers and observe that efficiency of Yumizen H2500 over DxH800, DxH900, and XN9011 (XN10) is 44%, 32%, 18% respectively. We found that non-auto validated results on Yumizen H2500 were 5.3%, on DxH800 were 4.5%, on DxH900 were 6% and on XN9011 (XN10) were 7.0% due to flags generated by analyzers. We prepared smear slide of each non-auto validated samples and reviewed under the microscope. We found that sensitivity of Yumizen H2500, DxH800, DxH90 and XN9011 (XN10) was 91%, 67%, 83%, 80% respectively. Specificity of Yumizen H2500, DxH800, DxH900, and XN9011 (XN10) was 61%, 55%, 27%, 29% respectively. We will process more number of samples to understand the sensitivity and specificity of the flags of various analyzers. **Conclusion:** The result of this study shows that four parameters i.e. Efficiency, Slide review rate, Flag sensitivity, Flag specificity of the various analyzers plays important role for a laboratory to enhance the productivity of the laboratory

B-135

Performance Evaluation of the cobas u 701 System for Urinalysis

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Background: The automated cobas u 701 sediment microscopy system (Roche Diagnostics) uses imaging analysis for in vitro quantitative determination of erythrocytes (RBCs) and leukocytes (WBCs), semi-quantitative determination of squamous epithelial cells (SEC), bacteria and hyaline casts (HYA), and qualitative determination of non-squamous epithelial cells, pathological casts, crystals, yeasts, mucus and sperm in urine. We evaluated the system's analytical performance, including precision, recovery of semi-quantitative range borders, carry-over, and method comparison versus conventional KOVA® microscopy.

Methods: Method comparison according to CLSI guidelines was performed at three sites using 689 residual, de-identified urine samples from individuals aged 0-98 years; manufacturer's instructions were followed for each method. Precision was assessed by calculating standard deviations (SDs) or coefficients of variation (CVs) within acceptable agreement rates. Recovery of defined concentration ranges for HYA, bacteria and SEC was assessed by diluting high-positive samples and measuring in triplicate on the cobas u 701 system and predicate methods. The cobas u 701 system was tested for sample carry-over using the Broughton model with subsequent measurements of high-positive and negative samples. Reference values were estimated for all parameters using residual routine urine samples from healthy individuals.

Results: Repeatability/intermediate precision estimates were all within manufacturer acceptance limits. In low concentration samples, SDs for RBCs and WBCs were all ≤1.1 cells/µL and ≤1.3 cells/µL limits, respectively. For quality controls in the pathological range (approximately 800 cells/µL for RBCs and 140 cells/µL for WBCs) intermediate CVs were ≤12.4%. Repeatability experiments for semi-quantitative and qualitative parameters confirmed reproducible recognition of negative and positive samples. No significant deviations for sample carry-over were found for any parameter tested. Method comparison of cobas u 701 system versus conventional KOVA microscopy demonstrated concordant results between study sites; regression slopes for combined results were 1.0 and 0.98, with negligible intercepts, for RBCs and WBCs, respectively. For semi-quantitative and qualitative parameters, negative percentage agreement rates were 88-100% and positive percentage agreement rates were 85-100%. Agreement rates for recovery at literature-based clinical cutoffs (RBCs, 10 cells/µL; WBCs, 25 cells/µL) were 99% for RBCs and 100% for WBCs. The cobas u 701 system allows users to relabel unidentified/misclassified particles. This functionality was used for all samples that had a clinically relevant deviation and the method comparison was repeated. A small improvement was observed, mainly in samples with a high rate of RBC dysmorphology, for which the cobas u 701 system may falsely flag artefacts/clumps/yeasts. Among 621 residual samples of known age and gender, 99th percentiles were 7.9 cells/µL (both genders) for RBCs, and 9.9 cells/µL (males) and 13.2 cells/µL (females) for WBCs.

Conclusion: Analytical performance of the cobas u 701 system met expectations for a new urinalysis system. Agreement with visual microscopy, without requiring reclassification, offers improvements for routine workflow, and the imaging technology is a useful system for microscopic urinalysis. The cobas u 701 system standardizes the entire urinalysis procedure for microscopy, reduces operator intervention, and offers centralized result management.

Disclaimer: cobas u 701 system is not yet cleared or approved for use in the USA.

B-136

Comparison of the T-TAS 01 PL Assay with PFA-100 for Assessment of Primary Hemostatic Function

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Background: The T-TAS01 PL assay is a novel in vitro diagnostic flow chamber system that passes whole blood through a collagen-coated microcapillary bed at arterial shear stress to measure the platelet thrombus formation process. The test is used to assess overall primary hemostatic function. Results are reported as the area under the pressure-time curve (AUC). The PFA-100 assay uses collagen-epinephrine (CEPI) and collagen-ADP (CADP) coated apertures for the detection of platelet dysfunction. CADP is interpreted when CEPI is abnormal. The purpose of this study was to compare the performance of the two assays in patients with impaired primary hemostasis.

Methods: Healthy subjects, subjects taking ASA, and subjects with confirmed vWD or GT were enrolled according to an IRB-approved protocol at five investigational sites. Blood sample collection, testing, and result interpretation were performed according to the manufacturer's instructions. Healthy subjects were screened for possible primary hemostasis defects and confirmed to have normal primary hemostatic function.

Results: 252 subjects were enrolled. The T-TAS01 PL assay was more specific (p=0.029) than PFA-100 for identifying impaired primary hemostasis. The T-TAS01 PL assay was more sensitive for aspirin-induced platelet dysfunction (p=0.004) and had comparable sensitivity for vWD and GT (p>0.05). The overall percent agreement between the assays was 80.5% (203/252), primarily influenced by the lower sensitivity of PFA-100 in ASA subjects. The T-TAS01 PL assay results were highly correlated with vWF antigen, vWF activity, and factor VIII activity levels (Spearman's rho=0.55, 0.83, and 0.71, respectively, all p<0.01). Only CADP results and vWF activity (Spearman's rho=-0.47) were correlated for PFA-100.

Conclusion: The T-TAS01 PL assay is highly sensitive and specific for impaired primary hemostatic function, and AUC results decrease proportionally with the severity of impairment. T-TAS01 PL assay measurements may be useful for the assessment of overall primary hemostatic function in patients with bleeding tendencies or defects in primary hemostatic function.

Summary Data								
				T-TAS PL Assay		PFA-100 CEPI		
Group	N	Mean Age	% Female	Mean AUC	Sensitivity/ Specificity	Mean CT (s)	Sensitivity/ Specificity	Agreement
Healthy Controls	142	38	67.6%	381.5	95.8% (specificity)	127	89.4% (specificity)	88% (125/142)
ASA	82	67	34.1%	221.0	70.7% (sensitivity)	196	51.2% (sensitivity)	63% (52/82)
vWD	25	46	78.0%	149.3	72.0% (sensitivity)	261	80.0% (sensitivity)	92% (23/25)
GT	3	25	100%	7.1	100% (sensitivity)	300	100% (sensitivity)	100% (3/3)

B-137

Effect of Storage Temperature and Time on Cat Blood and Serum Analytes

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Background: Whole blood and serum samples of different species are often kept refrigerated or frozen to preserve samples for subsequent research purposes. Few studies have been done to assess the stability of blood and serum samples in cats. The aim of this study was to assess the stability of various analytes in whole blood and serum from cats.

Methods: A total of 27 clinical chemistry analytes in sera and 16 analytes in whole blood of at least 5 cats were assessed following storage. To acquire a baseline concentration, measurements were taken from fresh samples. Subsequent to determining the baseline measurements for whole blood, the whole blood analyte stability was assessed following 8 hours of room temperature and 3 days refrigerated (4°C) storage using a Siemens ADVIA 120 Hematology Analyzer. Subsequent to determining the baseline measurements for sera, serum chemistry analyte stability was assessed following 8 hours at room temperature and at least 30 days of frozen (-20°C and -70°C) storage using a Siemens 1800 Clinical Chemistry Analyzer. At each time point, the percent difference between the concentration of each analyte and the mean baseline concentrations was evaluated.

Results: Results show that the hematology analytes were stable for 8 hours at room temperature and up to 3 days at refrigerated storage condition (4°C) (%Diff is ≤ 20%). Serum analytes were stable for 8 hours at room temperature and on ice storage. In addition, the serum analytes were stable up to 30 days at -20°C and -70°C except for creatine kinase (CK), total bile acid (TBA), sorbitol dehydrogenase (SDH), and glutamate dehydrogenase (GLDH). The percent differences for CK, TBA, SDH, and GLDH were all greater than 20%.

Conclusion: It can be concluded that the blood samples were stable up to 3 days at 4°C and serum samples were stable up to 30 days at -20°C and -70°C. CK, TBA, SDH and GLDH were not stable and should be analyzed on the same day that the samples are received in the laboratory. This study helps us to define acceptable storage times and conditions that can be used to preserve samples collected from cats.

B-138

CD26+ Leukemic Stem Cells Identification as a Tool for Chronic Myeloid Leukemia Diagnosis

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Background

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by proliferation of immature myeloid cells in the peripheral blood (PB) and bone marrow (BM), maintaining their capacity to differentiate. The increase of myeloid precursors is due to an acquired genetic alteration of the hematopoetic stem cells that behave as leukemic stem cells (LSC). It is characterized by the chromosomal translocation t(9;22)(q34.1;q11.2), which results in the formation of the Philadelphia chromosome, containing the BCR-ABL1 fusion gene. Although cytogenetics analysis is commonly used for CML diagnosis, RT-qPCR is more sensitive and is the most used to monitor minimal residual disease. In 2019, Raspadori et al. described a flow cytometry protocol for CML diagnosis and identified the expression of CD26 as a marker for CML LSC in peripheral blood and bone marrow. The aim of the present study was to compare a flow cytometry protocol for CML investigation with the gold standard diagnostic method BCR-ABL PCR assay.

Methods

PB and BM samples received between April 2019 and January 2020 for CML investigation with medical order for flow cytometry and PCR for BCR-ABL assays were retrospectively analyzed. For flow cytometry, samples were processed according to protocol previously described by Raspadori et al. and stained with the following anti-human monoclonal antibodies: HLA-DR-FITC (G46-6), CD123-PE (9F5), CD34-PerCP-Cy5.5 (8G12), CD117PE-Cy7 (104D2), CD38-APC-H7(HB7), CD33 BV421 (WM53), CD45-V500 (2D1) from BD Biosciences and CD26-APC (BA5b) from Exbio. Acquisition was performed on a 3-lasers, 8-colors FACSCanto™ II flow cytometer (BD Bioscence). Analysis and quantification of CML LSC (CD34+/CD26+/CD38-) was performed using Infinicyt (Cytognos). BCR-ABL1 was identified by an in house routine one-step RT-qPCR using ΔΔCq method. The buffy coats were removed from EDTA-whole blood (8 mL) or bone marrow samples (4mL), nucleic acids extracted using Magna 96 (Roche). A one-step RT-qPCR QuantiNova Probe master mix (Qiagen) with primers and probes described by Gabert et al. 2003 (EAC) and by Pane et al. 1996 were used. BCR-ABL1 and ABL RNAs were co-amplified at Roche LightCycler 480II for e14a2/e13a2 (p210), e1a2 (p190), and e19a2 (p230) fusions. The degree of agreement between the test methods (flow cytometry) and the comparative methods (RT-qPCR for BCR-ABL) was quantified using Kappa statistics.

Results

In this period, 21 samples from different patients were received for CML investigation with medical order for flow cytometry and BCR-ABL assays. Ten samples were from PB and 11 from BM. In 10 samples (3 BM and 7 PB), flow cytometry assay did not show a CD26+ CML LSC population, and BCR/ABL PCR assay resulted negative. In 11 samples (8 bone marrows and 3 peripheral blood), a CD26+ CML LSC population was identified by flow cytometry and BCR-ABL PCR assay resulted positive. There were no discordant results. The degree of agreement between flow cytometry and RT-qPCR for BCR-ABL was perfect (kappa=1).

Conclusions

Our data are in accordance with the results previously described by Raspadori et al. and identification of a CD26+ CML LSC population by flow cytometry may be a diagnostic tool for CML when a BCR-ABL PCR assay is not available.

B-139

Plasma Extracellular Vesicles as a Potential Biomarker of Disease Biology in Multiple Myeloma

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Background: Multiple myeloma (MM) is a devastating clonal plasma cell proliferative disorder responsible for over 13,000 deaths per year in the US. It is always preceded by the presence of pre-malignant clonal plasma cells termed monoclonal gammopathy of undetermined significance (MGUS) or a more advanced stage termed smoldering multiple myeloma (SMM). Both, MGUS and SMM, require continuous clinical monitoring to assess for their development of malignant plasma cells that results in progression to MM. However, no single clinical or laboratory feature can distinguish between the presence of premalignant or malignant clonal plasma cells in patients. As a result, MGUS and SMM patients who have already started to develop malignant clonal plasma cells progress to MM in between their scheduled clinical monitoring visits with organ damage such as lytic bone disease and/or renal insufficiency that adversely affects their survival. Thus, novel biomarkers which reflect the presence of malignant clonal plasma cells and identify patients who are destined to rapidly progress are needed in the clinic. Extracellular vesicles (EVs), including exosomes and microvesicles, are a promising source of circulating biomarkers and have shown clinical value in cancer diagnostics. The objective of this study was to: 1) determine the feasibility of assessing protein abundance from isolated plasma exosomes and microvesicles, referred to from now on as small EVs (SEVs) and large EVs (LEVs) respectively, in patients with MGUS and MM and 2) evaluate its value in providing potential biomarkers that reflect the presence of malignant clonal plasma cells as well as their prognostic disease biology. **Methods**: EVs were isolated from a total of 21 patients (MGUS : n=10, MM: n= 11) via size exclusion chromatography (SEC) using 1ml of platelet poor plasma. Isolated EVs were pelleted with ultracentrifugation at 20,000 x g for LEVs and 100,000 x g for SEVs. LEV and SEV pellets were digested with trypsin and the resulting peptides were analyzed by nano-LC-MS/MS on an Orbitrap Fusion Lumos mass spectrometer. Raw files were searched in MaxQuant software version 1.6.7 and data was analyzed using custom R scripts. Clinical correlatives from the patients in this study were abstracted form their electronic medical records. **Results**: In total, 2794 and 2083 proteins were identified in the LEV and SEV fractions respectively. A differential comparison between the MGUS and MM groups revealed 15 proteins with significantly altered abundance in SEVs and 13 proteins with altered abundance in LEVs. Interestingly, CD71(transferrin receptor) was upregulated in the MM group in both SEVs (log2FC = 2.6, q=.046) and LEVs (log2FC = 4.8, q= 0.029). An additional comparison of individuals with standard vs high cytogenetic risk (standard risk: n=5, high risk: n=5) identified 57and 44 significantly altered proteins in SEVs and LEVs respectively, indicating a significant association between EV protein abundance and cytogenetic risk. **Conclusion**: Assessment of plasma EVs in patients with plasma cell disorders is feasible and promises to be a reservoir of circulating biomarkers reflective of the presence of malignant clonal plasma cells and their prognostic disease biology.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Lipids & Cardiovascular

B-142

High HDL and Low Total Antioxidant Capacity are Predictors of Retinopathy in Type 2 Diabetes Mellitus Patients: A Case Control Study of the Cape Coast Metropolis

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Background: Diabetes mellitus is becoming an epidemic disease in Sub-Saharan Africa. Diabetes mellitus causes vision loss due to diabetic retinopathy (DR), which can be largely delayed in its onset or progression and even preventable if the potential risk factors for the disease are identified early and the appropriate therapy and lifestyle changes are made. This study investigated dyslipidaemia associated oxidative stress in Type 2 Diabetes Mellitus (T2DM) patients with retinopathy in the Cape Coast metropolis. **Methods:** This case-control study recruited 90 participants comprising 30 healthy non-diabetic participants, 30 T2DM participants with DR and 30 T2DM participants without DR. A well-structured questionnaire was administered to every participant to obtain information on their demographics and clinical history. All participants had both eyes screened for retinopathy with fundoscopy and then blood samples were drawn for assessment of conventional lipid profile [high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG)], fasting blood glucose (FBG) lipoprotein (a) levels, total antioxidant capacity (TAC) and oxidized LDL (Ox-LDL). **Results:** Total cholesterol (p=0.009), LDL-cholesterol (p=0.010), HDL-cholesterol (p=0.025), FBG (p=0.001) and serum lipoprotein (a) (p=0.002) were significantly higher in T2DM patients with retinopathy compared to non-diabetic healthy controls. Serum ox-LDL (p=0.140) and triglycerides (p=0551) were slightly raised in the T2DM participants with DR than in the T2DM participants without DR. Ox LDL showed a significant correlation with triglyceride (r=0.288; p=0.007) and BMI (r=0.345; p=0.007). Multivariate logistic regression analysis identified total antioxidant capacity (B=-0.05; SE=0.002; Exp (b)=1.005; p=0.008) HDL (B=-11.588; SE=4.48; Exp (b)=0.000; p=0.010), duration of diabetes (B=-0.386; SE=0.151; Exp (b)=0.680; p=0.011) and diastolic pressure (B=-0.02; SE=0.93; Exp (b)=0.818; p=0.032) as significant determinants of DR. ROC curves showed that high HDL-cholesterol (AUC=0.650; p=0.046, SE=0.071) and low TAC levels (AUC=0.783; p<0.001; SE=0.059) are significant predictors of DR. **Conclusio**n: Dyslipidaemia and oxidative stress depicted by high HDL and low total antioxidant capacity are significant predictors of retinopathy among our participants. Foods and drugs that boost antioxidants and HDL levels should be recommended to diabetes mellitus patients as well as reducing the intake of foods excessively rich in triglycerides.

B-147

Novel Cholesterol Efflux Assay using Immobilized Liposome-Bound Gel Beads: Confirmation and Improvement for Application in Clinical Laboratory

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Background The mortality of cardiovascular disease (CVD) has been increasing in many countries. High-density lipoprotein cholesterol (HDL-C) level has been used as a risk marker of CVD for a long time. However, some reports showed that HDL-C does not always predict the risk because it reflects just the quantity of HDL. Cholesterol efflux capacity (CEC) is widely known as one of the atheroprotective functions of HDL. Since CEC has already been confirmed to be a better risk marker of CVD, it is getting a lot of attention.To date, CEC has been measured using artificially-prepared foam cell composed of cultured macrophage and ^{3}H-cholesterol. However, this conventional method is not suitable for use in clinical laboratories because of the lack of simplicity and safety. Recently, we reported a novel CEC assay, immobilized liposome-bound gel beads (ILG) method. The ILG, which is alternative of foam cell, comprising gel beads and fluorescently-labeled cholesterol (BODIPY-cholesterol) instead of macrophage and ^{3}H-labeled cholesterol, respectively. Previously, we already reported basic properties of ILG method such as its stability and HDL-C concentration-dependence as well as strong correlation of CEC measured by between ILG method and conventional method which uses THP-1-derived macrophage (Horiuchi et al. *Biosci. Rep.* 2018). To launch this method in a clinical setting, many studies remain to be conducted.In this study, we conducted three further investigations which focus on measurement in clinical laboratory: the effect of anticoagulants in blood collection tubes, the effect of interfering substances in blood, and shortening the measurement time.

Methods and Results Blood samples were obtained from healthy volunteers or residual samples at clinical laboratory in TMDU medical hospital. First, the effect of anticoagulant in blood-collecting tubes was investigated. There was no difference of CECs in between serum and plasmas obtained using three different types of blood collection tubes (with EDTA-2K, EDTA-2Na, and heparin). Then, the effect of interfering substances on fluorescent intensity (FI) measurement was confirmed using Interference Check A plus (Sysmex). Hemoglobin and chyle did not affect, whereas both of free and conjugated bilirubin greatly interfered FI (5 mg/dL bilirubin addition increased FI to approximately 5 times). Consistent with this result, CECs of high-bilirubin samples (> 3.0 mg/dL) showed higher than those of samples with normal bilirubin level (< 1.0 mg/dL) which have same HDL-C. Subtraction of basal FI measured using BODIPY-free ILG minimized the bilirubin interference. Although our previous study adopted 16 hour-incubation, it is too long to be used in clinical settings. We tried to shorten it to 4 hours using ILG containing double amount of BODIPY-cholesterol. CECs measured by 16 hour- and 4 hour-method were well correlated (r = 0.937).

Conclusion ILG method is useful in clinical practice in terms of a less affected property by anticoagulants and completion of measurement within several hours. Although FI measurement was greatly affected by bilirubin, it could be decreased by subtracting the background FI. Further studies such as confirmation of clinical significance for CEC estimated by ILG method are needed to achieve our goal for establishment of the better risk marker of CVD.

B-148

Dramatic Change of High-Density Lipoprotein Structure and Serum Amyloid A Distribution after Orthopedic Surgery

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Background: High-density lipoprotein (HDL) is well known as a multiple functionality particle which suppresses a progression of atherosclerosis by numerous epidemiological and experimental studies. However, HDL is more complicated heterogeneous lipoprotein and further receives modification to be converted into dysfunctional HDL under pathological conditions like diabetes, oxidative stress and inflammation. One of the most important factors which can remodel HDL is serum amyloid A (SAA). During inflammation, SAA is produced in the liver and its serum level increases more than 1000 times to basal level. Most of the produced SAA would bind to HDL. Furthermore, SAA displaces apolipoprotein A-I (apoA-I) in HDL, main component of HDL. Recently, interesting study has been reported in which mice with only brief elevation of SAA levels had increased atherosclerosis. However, association between HDL remodeling and rapid inflammation has not been fully understood. Here, we monitored a rapid change of SAA distribution in HDLs and structural change of HDLs obtained from patients who had surgical operations.

Methods: Whole blood samples from 13 patients who underwent orthopedic surgery and didn't have any other medical diseases as liver disease were collected on before and just after (POST) the surgery and post-operative days (POD) 1, 3 and 6 or 7. SAA and lipids concentrations in the patients' plasmas were measured. SAA and apoA-I levels and their distributions in HDL fractions isolated by ultracentrifugation were analyzed by Native-PAGE and western blotting. Particle sizes and surface charges in HDLs were also evaluated by HPLC and electrophoresis, respectively. This study was approved by the institutional research ethics committee of the Faculty of Medicine, Tokyo Medical and Dental University [M2016-049].

Results: Plasma SAA levels were increased dramatically after surgery. The peak points of plasma SAA levels in all patients were on POD3. SDS-PAGE analysis for HDL showed that the relative intensity of the apoA-I band decreased by 16.5% on POD3 and then recovered on POD6 or 7, while the relative intensity of SAA band increased by 21.6% on POD3 and returned to basal level on POD6 or 7. When the HPLC

profiles of HDLs on POD3 were compared with those on POST, the retention time of the peak top on POD3 was shorter, and the ratio of the retention times on POD3 to those on POST showed a slight negative correlation with plasma SAA levels on POD3 suggesting that the histogram of HDL particle size was shifted to larger size in a SAA level-dependent manner. Consistent with this result, the profile of western blotting following Native-PAGE using anti apoA-I antibody showed that small particle size of HDL was apparently disappeared on POD3 and SAA distribution in HDL was spread, especially, in larger HDL. The HDL on POD3 had a lower negative surface charge than HDLs on the other PODs.

Conclusion: Analyzing HDL samples from patients after orthopedic surgery, we revealed that rapid size remodeling and change of surface charge were observed in HDL corresponding to the increase and decrease of SAA levels over time in same individual.

B-149

Comparison of Three Calculation Methods of Low Density Lipoprotein-Cholesterol: Results from the Multi-Ethnic Study of Atherosclerosis

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Background: The 2018 American Heart Association/American College of Cardiology (AHA/ACC) guidelines indicate that low density lipoprotein-cholesterol (LDL-C) should be used to direct management of atherosclerotic cardiovascular disease (ASCVD), which highlights the need for accurate LDL-C reporting. Traditionally, the Friedewald equation (FLDL) is used to calculate LDL-C. Newer equations, such as the Martin/Hopkins LDL-C formula (HLDL) derived from the Very Large Database of Lipids study, and a calculation derived from a National Heart, Lung, and Blood Institute (NHLBI) database (NLDL) both use a variable factor to account for inter-individual variability in the ratio of triglyceride (TG) to very low density lipoprotein-cholesterol (VLDL-C), thus providing more accurate LDL-C estimations. However, there is concern that alternative calculations may not perform as well in predicting long term ASCVD risk, as previous risk prediction studies were based on FLDL. We examined differences in these three methods, and evaluated the impact on their association with ASCVD.

Methods: Participants from the Multi-Ethnic Study of Atherosclerosis (MESA), who were free of ASCVD at baseline (aged 40-75) and had TG < 400 mg/dL were studied (N=6701). Baseline clinical and laboratory findings and ASCVD incidents were documented over a median of 10 years follow-up. LDL-C levels estimated by FLDL, HLDL, and NLDL methods were compared on their associations with clinical ASCVD, and with coronary artery calcium (CAC) as a subclinical marker of ASCVD, using chi-square test.

Results: In individuals aged 40-75 without diabetes, LDL-C < 70 mg/dL is the threshold defined by AHA/ACC for exemption from ASCVD risk discussion. Out of the 6701 MESA participants at study baseline, FLDL identified 241 individuals with LDL-C < 70 mg/dL, while HLDL identified 193 and NLDL identified 202. Of these, 22 (9.1%) grouped by FLDL, 20 (10.4%) by HLDL, and 20 (9.9%) by NLDL were found to have ASCVD during the follow-up. Difference between the method groups was not statistically significant (P>0.05). More patients with LDL-C < 70 mg/dL by FLDL had CAC present (65, 26.9%) than patients grouped by HLDL (44, 22.8%) or NLDL (47, 23.2%). There were a few ASCVD patients classified as < 70 mg/dL by FLDL that would have been re-classified as ≥ 70 mg/dL using the alternative LDL-C methods (N=3 for HLDL and N=2 for NLDL). However, only 1 ASCVD patient was classified as < 70 mg/dL by HLDL but as ≥ 70 mg/dL by FLDL.

Conclusion: The alternative LDL-C methods may help rule in more low-risk individuals for further risk evaluation that was shown to benefit them. The concern that the HLDL and NLDL may not offer comparable performance to FLDL calculations appears to be unfounded.

B-150

Development of Liquid Chromatography Based Assay for Profiling of Very-Long Chain Fatty Acids in Human Plasma

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Background: Measurement of very-long chain fatty acids (VLCFA) has profound importance in the diagnostic evaluation of peroxisomal dysfunctions. In addition, increasing evidence suggests that VLCFA are also associated with the risk of atherosclerotic cardiovascular diseases. Limited number of laboratory offers its measurement, which is partly because of the need for GC-MS. However, the GC-MS measurement of VLCFA itself is time-consuming and relatively inefficient. The use of LC-based system is gaining more popularity in clinical laboratories. Therefore, we aimed to develop a simple LC-based assay for the measurement of VLCFA in plasma that can be adopted with either spectrophotometry or mass spectrometry as a detection system depending on the need and availability of resources in clinical laboratories.

Methods: Esterified VLCFA (FA20:0, FA22:0, FA24:0, and FA26:0) in the plasma were first saponified to release into free form and were derivatized with 2-nitrophenylhydrazine. Tricosanoic acid (FA23:0) was used as an internal standard. The labeled VLCFA are then extracted and analyzed by high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS). We applied this method to measure VLCFA in 429 healthy human volunteers and 112 hypertriglyceridemic plasma. The reference intervals of each species of VLCFA were determined and association of VLCFA with various cardiovascular risk factor were assessed.

Results: The inter- and intra-assay imprecision of the VLCFA assays were within 10% and limit of detection of 1 pmol. There was a significant correlation (r above 0.96) between HPLC and LC-MS methods. 95 percentile reference intervals (upper limit) of VLCFA was determined to be 40.7 μmol/L in healthy volunteers. There was a significant positive correlation between circulating VLCFA concentration and serum triglycerides level with Spearman's correlation coefficient (ρ) of 0.306 (p<0.0001, 95% CI 0.228 to 0.381). The mean concentration of all species of VLCFA - FA20:0, FA22:0, 24:0, and 26:0 were significantly elevated in hypertriglyceridemia compared to normotriglyceridemic individuals. Serum concentration of VLCFA was significantly positively correlated with various cardiovascular risk factors including age, fasting glucose, total cholesterol, LDL-C, non-high-density lipoprotein-cholesterol, and total FA concentration.

Conclusion: A simple, relatively rapid LC method was developed for the measurement of VLCFA species in human plasma. Either spectrophotometry or tandem mass spectrometry can be used as a detection system with satisfactory analytical performance. The concentration of circulating VLCFA was positively correlated with serum triglycerides.

B-151

The Biological Variability of Plasma Ceramides in Healthy Subjects

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Background: Ceramides are bioactive lipid species that support the structure of the plasma membrane and mediate numerous cell-signaling events in eukaryotic cells. Given that ceramide concentration constitutes a risk factor for several pathologies, understanding the biological variability within an individual, and within a population of healthy individuals, would improve current understanding of ceramides as a biomarker and aid in interpreting patient outcomes. In this study, we aimed to define the monthly intra- and inter-indiviudal biological variability of four ceramides linked with cardiovascular disease and insulin resistance in a healthy population. **Methods:** The study cohort included 24 healthy adults (10 male / 14 female) mean age was 32 years (range 22 - 47) and BMI was 25.2 kg/m^2 (range 19.7 - 31.2). Plasma from fasting (≥ 8 h) subjects was collected daily for five days, weekly for four weeks, and monthly for seven months (n=232 samples). Ceramides: N-palmitoyl-sphingosine (Cer[16:0])) N-stearoyl-sphingosine (Cer[18:0]), N-lignoceroyl-sphingosine (Cer[24:0]), and N-

nervonoyl-sphingosine (Cer[24:1]) were measured by LC/MS/MS. For our analysis, we used random-effects regression models to estimate each variance component (daily and monthly within-subject; between-subject), and each estimate of variability was computed from these components. We reported variance, standard deviation (SD), coefficient of variation (CV%) and proportion of total variance (between-subject, monthly within-subject, and daily within-subject), along with the critical difference, intraclass correlation coefficient (ICC), and index of individuality (IOI). The monthly within-subject critical difference is regarded as the reference change value (RCV). All analyses were conducted using SAS version 9.4 (SAS Institute, Inc.). **Results:** Ceramide monthly concentrations ranged as follows, Cer(16:0), 0.16-0.38 mcmol/L, Cer(18:0), 0.03-0.18 mcmol/L, Cer(24:0), 1.6-4.6 mcmol/L, and Cer(24:1), 0.4-1.1 mcmol/L. Monthly within-subject variation (CV%) was 4.7%, 9.1% , 9.2% and 8.2%, for Cer(16:0), Cer(18:0), Cer(24:0) and Cer(24:1), respectively. Within-subject daily variation was 8.5%, 18%, 8.1% and 11% for Cer(16:0), Cer(18:0), Cer(24:0) and Cer(24:1), respectively.The greatest source of variation was attributed to between-subject differences with CV%s of 16%, 25%, 17% and 19% for Cer(16:0), Cer(18:0), Cer(24:0) and Cer(24:1), respectively... The critical differences (RCV) were 0.07 mcmol/L for Cer(16:0), 0.04 mcmol/L for Cer(18:0), 1.07 mcmol/L for Cer(24:0) and 0.27 mcmol/L for Cer(24:1). The Index of individuality (IOI) values were 0.82 for Cer(16:0), 1.11 for Cer(18:0), 1.04 for Cer(24:0) and 1.0 for Cer(24:1). **Conclusions:** The greatest variation reported comes from between-subject variation for all the ceramide species. According to our measurements, the most stable ceramide species was Cer16:0 with the lowest within-subject and between-subject differences in monthly measurements and highest ICC. Overall, this study demonstrates that the variability of ceramide concentrations at different time points is minimal within individuals. The IOI values observed (>0.6 in all cases) indicate that the expected variation for a given patient are smaller than the variation between individuals, therefore comparison of a single measure to a reference value is appropriate. Additional studies on the change in ceramide concentrations following interventions are needed.

B-152

Performance Evaluation of the Atellica CH Trig_2 Assay

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Background: Measurement of triglycerides in human serum and plasma is used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, and other diseases involving lipid metabolism. The purpose of this investigation was to evaluate the analytical performance of the Atellica® CH Triglycerides_2 (Trig_2) Assay on the Atellica® CH Analyzer and compare it to the commercial Atellica® CH Trig Assay. The commercial Trig assay uses ADVIA® Chemistry Systems Trig reagent, whereas the Trig_2 assay will use Dimension® Clinical Chemistry System TGL reagent. The assay is a single-reagent endpoint reaction. Lipase converts triglycerides to glycerol and free fatty acids. Glycerol kinase converts glycerol to glycerol-3-phosphate using ATP. Glycerol-3-phosphate becomes oxidized by glycerol-3-phosphate oxidase, generating hydrogen peroxide. Hydrogen peroxide reacts with peroxidase to form a colored quinoneimine dye complex that is measured spectrophotometrically. **Methods**: Performance testing included precision, method comparison, detection capability, linearity, and extended range. Precision was run over 5 days with five replicates per sample per day, for a total of 25 replicates per sample. Limit of blank (LoB) and limit of detection (LoD) were run over 3 days with four samples and five replicates per sample, totaling 120 replicates. Extended range testing used a manually diluted sample compared to an onboard dilution. Linearity samples were made using a 9-level dilution series. Method comparison and precision were evaluated based on CLSI documents EP09-A3 and EP15-A2, respectively. LoB, LoD, and extended range were analyzed using Microsoft Excel 2010. **Result**s: Results for the commercial Atellica CH Trig Assay were generated using ADVIA Trig_c reagent, and results for the Trig_2 assay were generated using Dimension TGL reagent. Within-lab precision for the Atellica CH Trig_2 Assay ranged from 0.9 to 1.5% CV. LoB for the Trig_2 assay was 1 mg/dL versus 3 mg/dL for the Trig assay, and LoD was 4 mg/dL for both the Trig_2 and Trig assays. The Trig_2 assay was linear from 15 to 1000 mg/dL, with extended range up to 10,000 mg/dL, whereas the Trig assay is linear from 10 to 550 mg/dL, with extended range up to 1100 mg/dL. Trig_2 method comparison versus the Dimension EXL™ assay yielded a slope of 1.06 and y-intercept of -7.8 mg/dL using a weighted least squares regression and r of 0.99, with n = 20 samples. **Conclusions**: The Atellica CH Trig_2 Assay tested on the Atellica CH Analyzer demonstrated acceptable precision, method comparison, and detection capability when compared to the on-market Atellica CH Trig Assay. The Trig_2 assay has an increased measuring interval and nearly 10 times the extended measuring interval compared to the commercial Atellica CH Trig Assay. *Product under development. Not available for commercial use or sale.

HOOD05162003064871

B-154

Blood Flow-Induced Dysregulation of TRAIL Expression Contributes to Human Carotid Atherosclerotic Plaque Instability: A Matter of Location?

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Background: Plasma levels of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), is significantly lower for patients with atherosclerosis. Low flow and shear stress in downstream areas within the dynamic atherosclerotic plaque microenvironment make it vulnerable to rupture. We previously showed that the presence of signaling molecules which render plaques susceptible to rupture, is prevalent downstream to flow. Our objective was to determine if there is an association between the expression of TRAIL and other molecular factors of vascular remodeling in human plaques along longitudinal-axial direction, upstream, and downstream from the stenosis, as shear stress varies dramatically. **Methods:** Carotid endarterectomy (CEA) specimens from 40 consecutive patients collected with flow-direction specifically marked, were randomly divided into two groups. Group A plaques were immunohistochemically analyzed along entire plaque length. Group B plaques were divided in half at the point of maximal stenosis, and classified as either "upstream" or "downstream", relative to flow. Next, total RNA was isolated from group B specimens for RT-PCR studies. **Results:** The mean age of the study patients (m=21, f=19) was 74.6±9.3 years**.** Of the 40 CEA plaques, 16 were from the right side; 15 were from symptomatic patients; 14 had >85% stenosis, 8 contained between 90-99% stenosis. Significantly lower TRAIL expression ($p<0.05$) was observed in downstream areas of the plaque as compared to upstream parts with high flow/high shear stress, where vascular smooth muscle cells are predominant. Fewer endothelial cells stained for endothelial nitric oxide synthase in the downstream areas as compared to upstream areas, while inducible NOS expression was predominant in areas with inflammation ($p<0.01$). CD68+ macrophages and T lymphocytes, were significantly higher in downstream areas of the plaque as compared to upstream areas ($p<0.01$). Osteopontin was augmented upstream as compared to the downstream regions, however the difference was not significant. **Conclusions:** The data provide *in vivo* evidence of mechanistic links between blood flow, inflammation, vascular remodeling, and plaque vulnerability in carotid atherosclerotic disease.

B-155

The Interference of Nonfasting State for Evaluation of the Lipid Profile according to the NCEP Guidelines

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Background: According to the National Cholesterol Education Program (NCEP) guidelines the blood sample collection for lipid profile should be done in fasting state. The standard lipid profile involving measurement of triglycerides and the indirect calculation of LDL cholesterol requires a 9 to 12-hour fast. Recently, some articles have been published suggesting that there is no need for fasting for blood collection in order to assess the lipid profile. The objective of this work was to study retrospectively the standard lipid profile observed in a laboratory routine among patients who fulfilled the fasting interval recommended by the NCEP and those who collected blood in the nonfasting state in a large private laboratory.

Methods: The standard lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol, VLDL-cholesterol and triglycerides) was evaluated in 231,977 outpatients. In this group 217,078 patients (93.5%) informed that they had fulfilled the recommended fasting interval and 14,902 patients (6.5%) reported that they had not followed the recommendation due to a medical recommendation or because they did not pay attention to this detail. Despite the warning about the possibility of changes in the result of the lipid profile, these patients opted to maintain the blood collection. The total cholesterol, HDL-C, LDL-C and triglycerides measurements were carried out on the Roche 8000 analyzer (Roche Diagnostics GmbH, Germany) using reagents from Roche. The LDL-C and non-HDL-C were indirectly calculated.

Results: The observed values of the standard lipid profile (mean ± standard deviation) with and without fasting were, respectively: Total cholesterol: 185 ± 39 mg/dL and 185±40 mg/dL; HDL-cholesterol: 55 ± 16 mg/dL and 55 ± 16 mg/dL; LDL-cholester-

ol: 109 ± 34 mg/dL and 109 ± 34 mg/dL; Non-HDL-cholesterol: 130 ± 39 mg/dL and 130 ± 39 mg/dL; VLDL-cholesterol: 21 ± 9 mg/dL and 21 ± 8 mg/dL; Triglycerides: 110 ± 73 mg/dL and 111 ± 84 mg/dL.

In this group, a significant difference was observed only for triglycerides.

Conclusion: Currently some scientific societies around the world are changing their guidelines towards a consensus on measuring a lipid profile in the nonfasting state aiming an attempt to simplify the blood sampling procedure for patients, laboratories, and clinicians. Our data demonstrate that a 9 to 12 hour fast is necessary for the evaluation the lipid profile and for the correct clinical interpretation of the results according to the recommendations of the NCEP guidelines.

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Smoking Adversely Affects Lipoprotein Profile by Causing Specific Increase in Atherogenic Small, Dense LDL-C Level

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Background: CVD is a major cause of death and disability. Significant CVD risk factors include age, gender, hypertension, smoking, diabetes, increased low-density cholesterol (LDL) and decreased high density lipoprotein cholesterol (HDL-C). Our goal in this large population study in Japan was to ascertain the relationships of smoking with other CVD risk factors, especially LDL-C, HDL, small dense LDL-C (sdLDL-C), and triglycerides (TG).

Methods: A total of 34,497 Japanese men and women, mean age 51 years, were recruited for this study as part of their annual health examination. All CVD risk factors were assessed. Serum total cholesterol, triglycerides (TG), HDL-C, sdLDL-C, and direct LDL-C were measured by standardized automated analysis after an overnight fast. Both univariate and multivariate analysis were carried to assess interrelationships of these parameters with smoking and other CVD risk factors.

Results: In both men and women, serum levels of TG and sdLDL-C were significantly ($p<0.01$) higher, and levels of LDL-C and HDL-C were significantly lower ($p<0.01$) in current smokers than in ex-smokers or non-smokers. Similarly, sdLDL-C levels in ex-smokers were in the middle, and significantly higher than those in non-smokers in men. Serum HDL-C levels in ex-smokers were higher than those in current smokers and were similar to those in female non-smokers and rather higher than those in male non-smokers.

Conclusion: Our data are consistent with the concept that current and prior cigarette smoking is associated with dyslipidemia consisting of elevated TG levels > 150 mg/dL, decreased HDL-C < 40 mg/dL, and increased sdLDL-C > 40 mg/dL. Therefore, smoking not only causes endothelial dysfunction, but also promotes dyslipidemia, providing a further reason to promote smoking cessation.

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Evaluation of Cholesterol Uptake and Efflux by Red Blood Cells

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Background: Quantifications of cholesterol in lipoproteins like low-density lipoprotein and high-density lipoprotein (HDL) are routinely used to predict a risk of atherosclerosis. Although very useful and reliable, these traditional biomarkers do not always fully reflect the risk of cardiovascular events. On the other hand, red blood cells (RBC), which occupy almost half of the total blood volume, carry large quantities of free cholesterol in their plasma membrane. Recent studies and our previous study demonstrated that RBC could also participate in reverse cholesterol transport system. Moreover, we showed that RBC transferred large quantities of cholesterol to plasma in a temperature-, energy- and time-dependent manner when RBC were mixed with plasma. Therefore, evaluation of this cholesterol transferring capacity by RBC might give us an important information for understanding the mechanisms of atherosclerotic plaque initiation and progression. Moreover, the factors relating the cholesterol transfer between RBC and plasma are poorly elucidated.

Methods: Human blood samples were collected from healthy volunteers, all participants provided informed written consent. We developed a cholesterol uptake assay using ^{3}H-cholesterol and four types of cholesterol carriers (apolipoprotein A-I (apoA-I), HDL, plasma, or methyl-β-cyclodextrin complexed with cholesterol (MβCD)). Packed RBC were mixed with each of cholesterol carrier labeled with ^{3}H-cholesterol, aliquoted into 96-well plate and incubated at 37°C for 0.5 - 3 hours. After centrifugation, supernatant was collected. The radioactivity in both supernatant and RBC lysates was measured to calculate the uptake rate (%). To evaluate the amount of cholesterol transferred from RBC, ^{3}H-cholesterol-labelled RBC were mixed with cholesterol acceptors (apoA-I, HDL or PBS as a control). After incubation at 37°C for 0.5 - 3 hours and centrifugation, the radioactivity in both supernatant and RBC lysates were measured to calculate the cholesterol efflux rate (%) from RBC.

Results: The cholesterol uptake by RBC was time-dependent and saturable for all carriers. When MβCD and apoA-I were used as cholesterol carries, RBC took up approx. 80% of cholesterol whereas only 20% was taken up when HDL or plasma were used as carriers. In cholesterol efflux assay, the RBC transferred cholesterol in a dose- and time-dependent manner when HDL was used as a cholesterol acceptor. However, cholesterol efflux to apoA-I was low and not dose- or time-dependent. When RBC were mixed with vanadate, an inhibitor of several of ATP-binding cassette (ABC) transporters, cholesterol uptake was decreased while cholesterol efflux rate was slightly increased. Similarly, cholesterol uptake was dramatically suppressed by Probucol, ABC transporter A1 inhibitor, while Probucol induced the cholesterol efflux from RBC to both apoA-I and HDL.

Conclusion: RBC actively participate in systemic lipoprotein metabolism by transferring cholesterol bidirectionally between RBC and plasma. Our study also revealed that some ABC transporters could be involved in the cholesterol transfer by RBC. We established cholesterol uptake assay and cholesterol efflux assay for evaluation of cholesterol transfer by RBC. Although further validation test and clinical study are needed, these assays would be available for novel biomarker for predicting the risk of atherosclerosis.

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Circulating Fibrillin-1 Fragments as Candidate Biomarkers for Aneurysm: Evaluation of Sandwich ELISAs

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Background: Blood-based biomarkers are needed to better identify individuals at risk for disease, to stratify risk for disease, and to monitor results of disease treatments. Circulating fibrillin-1 fragments have been proposed as candidate biomarkers for thoracic aortic aneurysm and dissection and for acute spontaneous cerebral artery dissection. Aneurysm and dissection are currently managed primarily by advanced imaging technologies. Because these conditions can lead to premature death, additional tools for identifying and stratifying risk are desirable.

Fibrillin-1 is the major protein component of the microfibrils associated with all elastic fibers. Aortic aneurysm occurs when the aortic wall undergoes changes and expands in size. A hallmark pathological feature of aortic aneurysm is fragmentation of the elastic lamellae. Aortic aneurysm and dissection are prominent features of the Marfan syndrome, which is caused by mutations in FBN1 (the gene for fibrillin-1). Both tissue location and human genetics support circulating fibrillin-1 fragments as candidate biomarkers for aortic aneurysm and dissection. In this study, we investigated the ability of our laboratory and commercially-available antibodies to detect native fibrillin-1 and the circulating fibrillin-1 fragments that may serve as biomarkers.

Methods: Circulating fibrillin-1 fragments, identified by monoclonal antibodies (mAbs) 15, 26, 78, and 201, have been proposed as biomarkers for aortic aneurysm and dissection. The main objectives of this study were to compare selected fibrillin-1 ELISAs and to characterize plasma fibrillin-1 fragments. Six commercially available fibrillin-1 ELISA kits were compared with our laboratory ELISAs using mAbs 15, 26, 78, and 201. Recombinant fibrillin-1 (rF11), produced in human 293 cells and known to be folded and functional, was tested in each of the six commercially available kits. Although the components supplied in each kit yielded good standard curves, there was no binding to rF11, indicating that the selected kits do not recognize native fibrillin-1. MAbs 15, 26, 78, and 201 were shown to bind to native fibrillin-1, and all epitopes except for the one recognized by mAb 15 required intact disulfide bonds.

Results: Plasma samples containing circulating fibrillin-1 fragments recognized by mAbs 15, 26, 78, and 201 were tested using the six commercial kits. None of the kits yielded results comparable to mAbs 15, 26, 78 and 201. Immunoaffinity chromatogra-

phy using mAb 15 and mAb 201, followed by mass spectrometry, showed that circulating fibrillin-1 fragments are composed of fibrillin-1 aggregates containing peptides from both the N- and C-terminal regions of fibrillin-1.

Conclusion: These novel findings indicate that circulating fibrillin-1 fragments, that contain epitopes for mAb 15 or for mAb 201, are most likely derived from tissue microfibrils rather than from newly synthesized fibrillin-1. In addition, while mAbs 15, 26, 78, and 201 have been used to quantitate native fibrillin-1 in plasma, it is unlikely that the six selected ELISA kits can be used in a similar manner. This study provides new important information about fibrillin-1 fragments used as biomarkers for tissue degradation and about requirements for the reagents used to detect these biomarkers.

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Establishing a Reference Interval for Heart-Type Fatty Acid-Binding Protein in a US Population

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Background: Heart-type fatty acid-binding protein (H-FABP) has been shown to be an effective, early marker of acute coronary syndrome (ACS), detectable as early as 30 minutes following an ischemic event. H-FABP can expand the toolkit in diagnostic sensitivity for ACS in conjunction with existing markers such as troponin. In order to interpret clinically relevant concentrations of H-FABP. a reference interval was established by measuring serum samples from the AACC Universal Sample Bank on the Cobas c502 (Roche Diagnostics) using the Randox immunoturbidimetric assay (Randox Laboratories Ltd, Crumlin, N. Ireland, UK). **Method:** Two hundred and fifty-four serum samples were acquired from the AACC Universal Sample Bank and analyzed using the Randox H-FABP immunoturbidimetric assay on the Cobas c502. Donor samples were screened to rule out risk factors for heart disease or pre-existing heart conditions. The sample set was comprised of 119 males and 135 females with an age range of 18 to 72 years old. Samples were received frozen. Small groups of samples were thawed for 30 minutes, aliquoted into new tubes, and lightly vortexed before immediately analyzing. During this process, samples were visually checked for any signs of potential interferants. Statistical analysis was performed following CLSI guidelines EP28-A3 to establish a reference interval using a non-parametric approach, after outlier elimination using the Tukey method. Further statistical analysis was performed to compare gender means using the Student's t-test and the correlation between age and H-FABP concentration by regression analysis. **Results:** The measured interval of H-FABP in the sample population was between 0.18 ng/mL and 17.37 ng/mL. Nine outliers were removed by the Tukey method and the combined non-parametric reference interval derived using this dataset was 0.52 to 6.58 ng/mL, with a mean of 3.10 ng/mL. When segregated by gender, the male reference interval was 0.52 to 7.13 ng/mL, the female was 0.48 to 6.31 ng/mL. Statistical comparison between genders showed no significant differences with the mean of the female population at 2.95 ng/mL and the male population at 3.28 ng/mL (p=NS). There was no significant effect of age on H-FABP concentration, however an insignificant upward trend was noted in older individuals (R^2=0.0128). **Conclusion:** Results were consistent with Randox manufacturer guidelines for the assay, with a 95% confidence mean of 3.55 ng/mL. No significant difference between genders, or correlation with age, suggests grouped reference intervals are unnecessary, however, further work could be performed for verification using larger sample sizes.

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The Utility of Remnant Lipoprotein as a Cardiovascular Risk Biomarker

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Background: Remnant lipoprotein cholesterol (RLC) has been touted as a new cardiovascular disease (CVD) risk biomarker [RLC = Total Cholesterol (TC) - HDL cholesterol (HDL) - LDL cholesterol (LDL)]. LDL and non-HDL cholesterol (non-HDL = TC - HDL) are also CVD risk markers. We evaluated the concordance between RLC, directly measured LDL (dLDL), Friedewald calculated LDL (cLDL) and non-HDL. **Methods:** In our hospital, the lipid panel comprises of total cholesterol (TC), HDL cholesterol (HDL), triglycerides (TG) and dLDL instead of cLDL. From 101,841 lipid panel results assessed on the Cobas 8000 auto-analyser platform (Roche Diagnostics, Singapore) between 2017-2019, we calculated RLC and cLDL. 409 samples were excluded for TG>4.5mmol/L and 31 samples were excluded for cLDL<0mmol/L (final N = 101,401 samples). Statistical analysis was performed using MedCalc software v18.11.3 (MedCalc, Ostend, Belgium). Our laboratory is a College of American Pathologists (CAP) accredited laboratory. As this work was part of routine evaluation of laboratory assays, no institutional review board approval was required. **Results:** The correlation between dLDL/cLDL, cLDL/non-HDL and dLDL/non HDL in our population was satisfactory (r = 0.98/0.95/0.96 respectively). When RLC was derived using cLDL, the RLC ranged from 0.09-2.07mmol/L, while RLC derived using dLDL ranged from 0.0-3.58mmol/L. RLC showed poor correlation with other established lipid biomarkers (cLDL, dLDL and non-HDL) in both situations (see Table 1). When RLC was calculated using dLDL, 24,184 samples (23.8%) were negative. ROC analysis showed that a TG of 1.07mmol/L had a sensitivity/specificity of 75.27/78.30 for detectable RLC (AUC = 0.85). **Conclusion:** The negative bias from three separate measurements, coupled with a general underestimation of dLDL by cLDL, may have contributed to the negative RLC values. Further research is clearly required to see if RLC performs as well as LDL/non-HDL in the prediction of CVD.

Table 1. Correlation of Remnant Lipoprotein Cholesterol (RLC) with other Lipid Biomarkers

RLC derived using cLDL	Correlation coefficient (95% CI)	Intercept	Slope
cLDL	0.091 (0.0852-0.0974)	-1.83	1.06
dLDL	0.170 (0.164-0.176)	-1.67	0.83
non-HDL	0.387 (0.3821-0.3925)	-0.78	0.48
RLC derived using dLDL	**Correlation coefficient (95% CI)**	**Intercept**	**Slope**
cLDL	0.042 (0.0350-0.0491)	-10.83	4.83
dLDL	0.01 (0.0028-0.0169)	-56.82	20.92
non-HDL	0.258 (0.2513-0.2644)	-2.16	0.81

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Diverse Short-Term Natriuretic Response in Acute Decompensated Heart Failure Patients Undergoing Entresto™ Treatment

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Background: A heart failure (HF) drug named Entresto™, which contains an inhibitor of protease neprilysin (NEP), is currently being introduced into clinical practice for the treatment of chronic and acute decompensated HF (ADHF), with a more pronounced effect in the latter pathology (PIONEER-HF trial). As the bioactive form of B-type natriuretic peptide (BNP) is a known NEP substrate, Entresto™ is believed to be beneficial for HF patients being able to decrease the NEP-mediated degradation of the bioactive BNP. However, the details of this impact are yet to be explored. The BNP pool in the bloodstream is composed of the BNP precursor proBNP and various truncated forms of BNP, some of which retain physiological activity. This complexity makes the direct measurement of bioactive BNP in the circulation virtually impossible. We have previously described one proteolytic form, BNP-neo17, which is generated from BNP by NEP cleavage at 17-18 aar. Since BNP-neo17 is NEP-dependent, it may reflect the impact of NEP inhibition on the bioactive BNP level.We evaluate the short-term dynamics of several BNP forms, including the novel BNP-neo17, in ADHF patients undergoing Entresto™ treatment.

Methods: 12 patients hospitalized for ADHF (NYHA functional class II-IV) received Entresto™ for 4 days after admission. Blood was sampled at the baseline and 4 hours after taking the drug on days 1, 2, 3, and 4. NT-proBNP was detected by the automated Roche Cobas e 411 analyzer and the total BNP level was measured by an in-house SES-BNP plate immunoassay. Meanwhile, BNP-neo17 was measured by an in-house sandwich immunoassay based on a rabbit recombinant antibody that was specific to the proteolytic neo-epitope (containing Arg 17) and the anti-BNP mAb 50E1 (HyTest Ltd.).

Results: The NT-proBNP levels on admission varied between 508.6 and 45430.0 ng/L (median 2245.0, IQR 1245.3 - 7274.0 ng/L). All 12 patients exhibited a decrease in NT-proBNP levels during the 4 days of treatment, which ranged between -29.8% and -82.3% (median -57.8%, IQR -43.5 - -65.2%). Total BNP levels on admission were in the range of 197.4 - 27072.0 ng/L (median 1923.2, IQR 987.3 - 4785.5 ng/L). A considerable increase in total BNP concentrations (up to 158.5%) was observed on the second day of Entresto™ therapy (median 21.6%, IQR 4.6-65.1%). However, this elevation was followed by a decrease in total BNP concentrations (up to two fold) on day 4 (median -39.1%, IQR -8.2 - -58.5%). The BNP-neo17 levels on admission varied from 0.8 to 96.7 ng/L (median 23.1, IQR 5.6-33.7 ng/L). Six patients exhibited a BNP-neo17 decrease. One patient demonstrated no change in the BNP-neo17 level, while successive fluctuations in biomarker concentration were observed in five of the patients throughout the treatment.

Conclusion: Our results demonstrate that while the NT-proBNP levels decreases in ADHF patients during Entresto™ treatment, the highly diverse dynamics of BNP-neo17 concentrations indicates that NEP inhibition does not lead to an expected pronounced drop in NEP-mediated degradation of BNP. We believe that monitoring the BNP-neo17 or other NEP-derived BNP forms during Entresto™ treatment might shed more light on the mechanisms underlying the effects of NEP-inhibition based therapy.

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A Compound Heterozygosity Mutation in ABCG8 Gene in a Child with Exuberant Eruptive Xanthomas and Hypercholesterolemia

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Background: Mutations in ABCG5 and ABCG8 genes have been associated with sitosterolemia, an autosomal recessive inherited disease characterized by hyper low-density (LDL-c) cholesterolemia and xanthomas. Possible pathophysiological mechanisms involve increased intestinal absorption and impaired sterols biliary secretion of phytosterols. **Case report**: We present a 10-years-old girl with exuberant eruptive xanthomas in the knees, ankles and buttocks since 7-years-old (figure 1). Initially, she was suspected as familial hypercholesterolemia (FH). Before the introduction of treatment, which included dietary changes, rosuvastatin 10 mg/day and ezetimibe 10 mg/day, baseline tests were assessed. Blood samples were collected after overnight fasting, and serum total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were assayed enzymatically. Genetic study was performed using a custom panel for lipid-associated genes, including LDL receptor, APO-B, PCSk-9, ABCG5, and ABCG8 genes. A compound heterozygosity mutation in ABCG8 gene (p.Trp361* and p.Ser609Thrfs*54), compatible with sitosterolemia, was detected. In the follow-up, LDL-c levels exhibited significantly reduction (LDL-c baseline 374mg/dL and after treatment LDL-c 97,2mg/dL) . **Conclusion**: Sitosterolemia is a rare recessive disorder that should be suspected in patients with eruptive xanthomas and hyper LDL-cholesterolemia. Ezetimibe, a NPC1L1 inhibitor, plays a pivotal role on intestinal absorption of sterols and has become the treatment of choice. Early treatment since childhood is important to avoid premature atherogenesis and unfavorable cardiovascular outcomes.

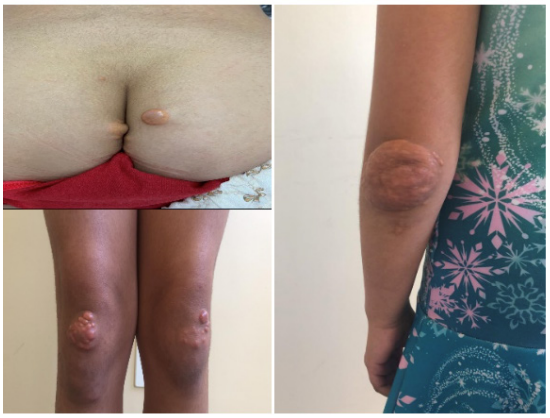

Figure 1:Presence of xanthomas in the knees, buttocks and elbows

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Demographic and Genetic Profile of Patients with Familial Chylomicronemia Syndrome Phenotype

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Background: Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disorder with loss of function mutations of lipoprotein lipase (LPL) resulting in hypertriglyceridemia and accumulation of chylomicrons in plasma and with the highest risk of acute pancreatitis. In FSC the main genetic mutations are homozygous LPL deficiency, homozygous loss of function mutations in APOC2, APOA5, GPIHBP1, LMF1 and G3PDH1 genes. Compound heterozygous mutations involving LPL and these genes should be considered in FCS. Due to the strong similarities of the phenotype between FCS and multifactorial chylomicronemia syndrome (MCS), the genetic study provides a clinical tool able to discriminate between most cases. **Objetive**: To describe the demographic and genetic profile of patients with FSC phenotype. **Methods:** This is a cross-sectional study with demographic and genetic analysis of patients with FSC phenotype referred to the dyslipidemia center, in the period from 2013 to 2020. Next-generation sequencing was performed with a painel of genes: ABCA1 AGPAT2 AKT2 APOA5 APOC2 BSCL2 CAV1 CFTR CIDEC CTRC CYP27A1 GPIHBP1 LIPA LIPE LMF1 LMNA LMNB2 LPL PLIN1 POLD1 PPARG PRSS1 PSMB8 SMPD1 SPINK1 ZMPSTE24. **Results:** A total of 20 patients performed genetic test: 12 children (<19 yrs. old) and 8 adults. A total of 12 had positive mutation (9 children and 3 adults). The demographic and genetic profile is showed in table 1. **Conclusion**: Despite a rare disease our lipid clinic allows us to identify different FSC genotypes including rare forms. Next-generation sequencing is likely to facilitate the diagnosis of FCS, since all the candidate genes can be tested in the same run. However, due to its cost and paucity in core laboratories, not all clinicians have access to genetic assessment.

Demographic and genetic profile of patients with familial chylomicronemia syndrome phenotype

Gender	Age (years)	Disease classification	Gene	Genomic location	Variation	Consequence	Patogenecity
Male	0.25	Homozygous	GPIHBP1	chr8:144.296.887	G > T	c.182-1G>T ENST00000622500	Patogenic
Female	1.0	Homozygous	GPIHBP1	chr8:144.296.887	G > T	chr8:144.296.887 G > T c.182-1G>T ENST00000622500	Patogenic
Male	4.0	Homozygous	LPL	chr8:19.813.381	G > A	p.Glu269Lys ENST00000650287	Probable patogenic
Female	5.0	Heterozygous	APOA5	chr11:116.661.264	G > T	p.Cys227* ENST00000227665	Probable patogenic
Female	5.0	Homozygous	APOA5	chr11:116.661.264	G > T	p.Cys227* ENST00000227665	Probable patogenic
Male	8.0	Homozygous	LMF1	chr16:920.035	G > A	p.Gln422* ENST00000262301	Patogenic
Male	15.0	Heterozygous	GPIHBP1	chr8:19.813.381	G > T	c.182-1G>T ENST00000622500	Patogenic
Female	15.0	Heterozygous	LPL	chr8:19.813.381	G > A	p.Asp231Asn ENST00000650287	Variant of uncertain significance
Female	18.0	Homozygous	LPL	chr8:19.813.381	G > A	p.Glu269Lys ENST00000650287	Likely patogenic
Median	5.0						
Female	34.0	Heterozygous	GPIHBP1	chr8:144.296.887	G > T	c.182-1G>T ENST00000622500	Patogenic
Male	37.0	Compound heterozygous, 2 affected genes	GPIHBP1	chr8:144.296.887	G > T	c.182-1G>T ENST00000622500	Patogenic
Male	37.0	Homozygous	GPIHBP1	chr8:144.296.887	G > T	c.182-1G>T ENST00000622500	Patogenic
Mean	35.0						

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Genetic and Clinical Profile of Children with Familial Hypercholesterolemia from a Lipid Center of Brazil Northeast

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Background: Familial hypercholesterolemia (FH) is an autosomal dominant disease, caused by mutations in the low density lipoprotein receptor (LDLR) gene, apolipoprotein B (APOB) gene or proprotein convertase subtilisin/kexin type 9 gene (PCSK9). The diagnosis of FH in children and adolescents is much more challenging, because early in life, the disease is asymptomatic. **Objective:** This study aimed to describe the genetic data obtained of children from HIPERCOL, Ceara, Brazil program. **Methods:** This cross-sectional study evaluated children with genetic diagnosis of FH from the cascade screening program from 2013 to 2019. The mutation detection was initially made by LDLR gene sequencing. If a disease causing mutation was not found, the PCSK9 (Exon 7) and APOB (part of exon 26) genes both considered as hotspots were

studied. In the case of a negative result in all three genes, the MLPA technique was used to search for deletions or insertions in the LDLR gene. Sanger sequencing was performed in the ABI3500xl sequencer and the sequences were analyzed with the software SeqMan. **Results:** We evaluated 43 children (17 female and 26 male), median age was 7.0 years old (min: 1; max: 18). A total of 24 were presented LDLR gene mutation (Table 1). A total of 18 children inherited the mutation from one parent and 6 children inherited the mutation from both parent (compound heterozygous). One of those children presented numerous xanthomas. The LDL levels were 3.31 mmol/L for simple heterozygous cases and 9.9 mmol/L for the compound heterozygous cases. **Conclusion:** Only mutations in receptor (LDLR) gene were present in all children. The simple heterozygous form was the most prevalent. The only clinical characteristic was high LDL-C levels. Physical signs were uncommon and most of children were asymptomatic.

Genetic profile of children with familial hypercholesterolemia

Location LDLR gene	Aminoacid change	N=24
Exon 4	p.Arg115Cys	3
Exon 5 e Exon 6	Duplication	1
Exon 7	p.Ser326Cys	4
Exon 7	p.Ser326Cys	1
Exon 8	p.Gly373Asp	4
Exon 8	C392Y(Cys392Tyr)	3
Exon 9	p.Arg406Trp	2
Exon 9	A431Ala431Thr	3
Exon 10	p.Val523Met	1
Exon 14	P699L(PRO699Leu)	1
Exon 15	p.Gln722*	1
Exon 16	p.lle792Metfs*137	3
Exon 16	L792MfsX136 (lle792MetfsX136)	1

B-169

Diagnostic Accuracy of TOSOH BNP for the Diagnosis and Assessment of Severity of Heart Failure in an All-Comers Population

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Background: Brain natriuretic peptide (BNP) is a cardiac hormone known to have diuretic, natriuretic, and vasodilatory effects as well as suppress sympathetic nervous and renin-e angiotensin-aldosterone systems. The release of BNP from the heart is increased in response to mechanic or neurohormonal stimulation of the heart, leading to a rise of BNP levels in the blood. Measurement of BNP concentration is utilized for assessment of the severity of heart failure (HF). In this study, BNP was validated at the currently recommended BNP cut-off of 100 pg/mL as recommended in the American Heart Association guidelines for acute HF. Clinical performance of the BNP assay as an aid in the diagnosis and assessment of the severity of heart failure was evaluated in patients presenting to the Emergency Department (ED) or doctor's office with clinical suspicion of new or exacerbated HF, with or without overt signs/symptoms where a clinician may order a natriuretic peptide test as part of the differential diagnosis (HF versus non-HF). A secondary objective was to explore the connection between BNP and NYHA stage, gender, age and source (ED or not).

Methods: Sample testing was performed with Tosoh ST AIA-PACK BNP (two-site immunoenzymometric assay) on a Tosoh AIA 2000 Analyzer. Diagnosis of HF or non-HF was determined by a blinded independent central adjudication panel, INOVA Health Care Services, in order to ensure standardization and accuracy of diagnoses per the 2013 ACCF/AHA Guidelines for Management of HF. Severity of HF (NYHA Classification I, II, III, IV) was also determined by the adjudication panel. A total of 932 patients were enrolled at 15 study sites; 829 patients were evaluable for analysis (ambulatory clinic, n=85; ED, n=744; 45% female; 36% age >75 years). Concordance of adjudication results with clinical diagnosis was examined.

Results: Using the traditional single cutoff of 100 pg/mL, the sensitivity of the ST AIA-PACK BNP assay is 86.9% (95% CI 83.2-89.9%) and the specificity is 70.2% (95% CI 65.7-74.2%). For the ED only population, the sensitivity is 88.8% (95% CI 85.0-91.7%) and the specificity is 69.3% (95% CI 64.6-73.6%). According to linear regression analysis, each one stage increase in NYHA corresponded to a 0.353 increase in the natural log of the BNP assay. Therefore, with each one stage increase in NYHA stage BNP level increases by 42%. **Conclusion:** In this study, for the first time, BNP performance in an all-comers population was evaluated. BNP was shown to be sensitive for the diagnosis and assessment of severity for heart failure. The BNP assay has demonstrated acceptable clinical performance as an aid in the diagnosis and assessment of the severity of heart failure in an all-comers population

B-170

Accuracy of Friedewald and Martin Calculated LDL in Patients with Monoclonal Gammopathy

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Background: Patients with monoclonal gammopathy are at risk of hyperlipidemia. Low-density lipoprotein cholesterol (LDL-C) is one of the primary markers for predicting and monitoring cardiovascular disease. However, Friedewald's formula and Martin calculator are the primary methods used by laboratories for the calculation of LDL when triglyceride (TG) is less than 400 mg/dL. We determined the accuracy of both Friedewald and Martin calculated LDL compared to direct LDL in known monoclonal gammopathy patients with TG less than 400 mg/dL.

Methods: We determined lipid profile panel, including direct LDL-C in twenty-three (23) known monoclonal gammopathy patients (18 males, 5 females; mean age was 63 ± 11 years), after which LDL was calculated using Friedewald's formula and Martin calculator. The bland-altman analysis was used to determine the agreement between direct LDL measurement and Friedewald's calculated LDL. Additionally, the agreement between Martin calculated LDL and measured LDL was determined. Correlation analyses between the different LDL methods were performed.

Results: We observed the overestimation of LDL with both Friedewald and Martin's calculation in approximately 70% of patients with monoclonal gammopathy. The mean of direct LDL, Friedewald, and Martin calculated LDL were 79.1 ± 40.6 mg/dL, 89.4 ± 41.3 mg/dL, and 94.0 ± 52.3 mg/d, respectively. A weak correlation was found between direct LDL and Martins calculated derived LDL ($r^2 = 0.60$), and Friedewald was 0.72. Of note is that the delta between direct LDL and Friedewald calculated LDL was between 1.8 mg/dL to 57 mg/dL, while Martin ranges between -65 mg/dL to 121 mg/dL. Also, using the Bland-Altman plot we observed bias of 10 and 14 with Friedewald and Martin when compared with direct LDL measurement, respectively. This suggests a systemic bias between both direct LDL and Friedewald and Martin's calculated LDL.

Conclusion: When compared with direct-LDL, both Martin and Friedewald are not reliable methods for determining the LDL levels of patients with monoclonal gammopathy.

B-171

Comparison of Siemens and Beckman High Sensitivity Troponin I Assays by Two Network Medical Centers

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Background: The fourth Universal Definition of Myocardial Infarction recommends high sensitivity troponin (hs-cTn) monitoring. For patients presenting with acute chest pain, history, physical examination, electrocardiogram (ECG), and biomarkers are pivotal in assessing patients with suspected acute coronary syndrome (ACS). Current practice involves serial measurements of cTn I or T, with concentration above the 99th percentile of the upper reference limit (URL) for a diagnosis of MI. Hs-cTn offers enhanced detection with whole number reporting with shorter time intervals. We compared the contemporary Siemens (TnI-Ultra Siemens) and Beckman (TnI Beckman) troponin I assays with the high sensitivity Siemens (hs TnI Siemens) and Beckman (hs TnI Beckman) troponin I assays for the patient population served by Wake Forest Baptist Health (WFBH) and High Point Medical Center (HPMC).

Methods: For comparing hs-cTnI assays, WFBH collected 174 lithium-heparin plasma samples, and HPMC collected 100 samples. In order to minimize possible false positive "flyers", an online protocol was used for WFBH samples. WFBH used Siemens Centaur TnI-Ultra and HPMC used Beckman DXi TnI. In parallel, these samples were also run on hs TnI Siemens and hs TnI Beckman at WFBH. Batch sizes were: 23/day for WFBH and 29/day HPMC. Precision and linearity studies were conducted for WFBH and HPMC. Interassay variability was minimized by running

all samples fresh with none being subject to cold storage prior to testing. Intraassay accuracy was assessed by repeating the three troponin I assays on spun serum. Data was analyzed using EP Evaluator.

Results: Linearity and precision agreed with the manufacturers' recommendations. Four correlation studies showed the following slopes, Y-intercepts and sample number: 1. WFBH TnI-Ultra Siemens vs hs TnI Siemens, 0.685, 34.2 and 174; 2. WFBH TnI-Ultra Siemens vs hs TnI Beckman, 0.409, 53.5, and 174; 3. WFBH hs TnI Siemens vs hs TnI Beckman, 0.602. 29.1, and 174; 4. High Point Contemporary TnI Beckman vs hs TnI Beckman 0.706, 7.5, and 100.

For WFBH, serum samples > 99th% URL categorized by hs TnI Beckman assay:93/174 (54%) of, while the TnI-Ultra Siemens and hs TnI Siemens, 87/174 (50%) and 58/174 (33%) respectively, based on the manufacturer's suggested unisex clinical cut-offs. Sensitivity and specificity were 95% and 51% hs TnI Beckman, 100% and 57% TnI-Ultra Siemens, and 68% and 70% hs TnI Siemens. For HPMC, serum samples ≥ 99th% URL categorized by hs TnI Beckman assay was 29/100 (29%) of, while the Beckman contemporary TnI assay, 22/100 (22%), based on the manufacturer's suggested sex-specific clinical cut-offs. Sensitivity and specificity at these cut-offs were 76% and 100% hs TnI Beckman, and 100% and 91% for Beckman Contemporary TnI.

Conclusion: Our findings indicated that the hs TnI Beckman assay will categorize more patient troponins at or above the 99th percentile of the URL than either TnI-Ultra Siemens or hs TnI Siemens assays. Adoption of the Beckman assay will harmonize hscTnI testing in our healthcare system. Siemens hs-cTnI assay identified a lower proportion of patients.

B-172

Evaluation of Triglycerides Levels after a Second Collection in Patients with Previous Very High Levels of Triglycerides

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Background: According several clinical studies high levels of triglycerides may induce atherosclerotic cardiovascular disease. Many factors can contribute to high triglyceride levels, including overweight and obesity, physical inactivity, cigarette smoking, excess alcohol intake, very high-carbohydrate diets, drugs (corticosteroids, protease inhibitors for HIV, beta-adrenergic blocking agents, estrogens), genetic factors and medical conditions such as diabetes and kidney disease. The triglycerides determinations may also be affected by biological and preanalytical variations. The aim of this study was to evaluate triglycerides variation following a collection of a new specimen in patients with high levels of triglycerides concentration.

Methods: This study analyzed retrospectively all lipid profile samples ordered in outpatient and inpatient settings during a 21-month period (January 2018 to September 2019) in a large private laboratory. The samples with high levels of triglycerides (higher than 1,000 mg/dL) were selected and a subsequent tracking of the next collection was performed. The triglycerides results of the new collection samples were then compared to the previous one. The triglycerides measurements were carried out on the Roche 8000 analyzer (Roche Diagnostics GmbH, Germany) using reagent from Roche.

Results: A total of 2,914,948 lipid profile samples were analyzed in the period and 1,612 (0.05%) samples presented high levels of triglycerides concentration (mean triglycerides 1,649±1,001 mg/dL, median age 46.5 (0-93 years), 98.9% aged more than 19 years, 78.4% male). A collection of a new specimen was ordered for 873 patients and 145 (16.6%) samples presented a normal level of triglycerides concentration (mean 125±30 mg/dL) in a median time period of 69 (6-574) days; 154 (17.6%) patients had persistent high level of triglycerides after a new collection (mean triglycerides concentration 2,035±1282) in a median time period of 101 (4-570) days; 574 (65.7%) patients had elevated triglycerides concentrations between 175 and 999 mg/dL after a collection of a new sample.

Conclusion: Most patients (82,3%) presented a fall in triglycerides concentration below 1,000 mg/dL after a collection of a new specimen. Most of these patients, however, had a triglycerides concentration above the upper limit interval on a new determination. Of note, 16,6% of patients presented normal triglycerides levels after a new collection, suggesting preanalytical issues or intense treatment. The remaining 17.6% cases that maintain high triglycerides levels possibly are at risk for complications such as pancreatitis due to high levels of triglycerides concentration.

B-175

Falsely Decreased Lipid Profile due to Monoclonal Paraprotein: A Korean Patient with Waldenstrom Macroglobulinemia

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Background: High levels of monoclonal paraprotein can interfere with lipid profile assays, resulting in artifactually decreased results of LDL-C, HDL-C, and total cholesterol. If this interference is neglected, this may result in the mismanagement of the patient because LDL-C and HDL-C are associated with increased and decreased risk of cardiovascular disease, respectively. We report a Korean patient with Waldenstrom's macroglubulinemia presenting with low levels of LDL-C, HDL-C, and total cholesterol**,** presumably due to interference of monoclonal paraprotein. **Methods (Underlying patient history):** A 58-year-old male with underlying diabetic retinopathy, macular edema, and cataract was admitted to our institution due to dizziness. Initial CBC results were Hb 6.3 (g/dL) - WBC 3780 (cells/mm^3) - Platelets 65 (x10^3/L), showing anemia and thrombocytopenia, and total protein 11.1 (g/dL), albumin 2.6 (g/dL) showing A/G ratio inversion. Further testing was undergone on the clinical suspicion of a malignant immunoproliferative disorder such as plasma cell myeloma. The patient showed increased IgM >5850 mg/dL, and free kappa and lambda light chain levels were 215 mg/L and 3.98 mg/L respectively. M-spike was detected in protein electrophoresis labs, 5.44 g/dL (51.8%) and 28.7% in the gamma regions of serum and urine respectively, while serum immuno-electrophoresis gave a result pointing to a monoclonal gammopathy; IgM, kappa type. A bone marrow study was conducted for diagnostic confirmation and was reported as hypercellular (cellularity 91-100%) marrow with diffuse infiltration of lymphoid cells, with a final diagnosis of R/O Lymphoplasmacytic Lymphoma, Waldenstrom's macroglobulinemia.**Results (Patient lipid profile history and clinical intervention):** The total cholesterol of the patient at first visit was a very low 28 mg/dL, and additional testing at a later date showed low levels of HDL-C and LDL-C (16 and 21 mg/dL respectively). After the patient's clinical history was made aware to the laboratory, subsequent total cholesterol results were reported as <50 mg/dL (due to difference in assay/instrument), with comments of possible interference due to paraprotein added for each result. Parallel testing conducted in the laboratory with clinical chemistry analyzers of 3 different manufacturers (Beckman Coulter, Atellica [Siemans], VITROS [Ortho Clinical Diagnostics]) all showed low total cholesterol. In order to determine the true values of the patient's lipid profile, supplemental lipoprotein electrophoresis was conducted, and the patient's samples were also referred to the Korea Center for Disease Control and Prevention (KCDC) for confirmation tests utilizing the Abell-Levy-Brodie-Kendall reference measurement procedure. **Conclusion:** We report a case of falsely low lipid profile due to suspected interference of monoclonal paraprotein. As total cholesterol, HDL-C, and LDL-C levels are critical to the risk evaluation of cardiovascular disease, clinical vigilance is required when direct methods of lipid testing give unusually low values, especially in hematological disorders with increased monoclonal paraprotein.

B-176

Confidence in Adopting hs-TnT Guidelines for Ruling out Suspected NSTEMI

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Background: For ruling out suspected non-ST elevation myocardial infarction (NSTEMI) in chest pain patients in the emergency department, the European Society of Cardiology (ESC) recommends a high-sensitivity troponin T (hs-TnT) cut-off below 5ng/L or below 12 ng/L plus a delta (zero-1 hour) of under 3ng/L. Confident adoption of these guidelines is contingent on good assay precision around these cut-points. There is paucity of data with respect to hsTnT assay precision below 12ng/L using a large cohort of actual patient samples.

Methods: In routine practice we test hs-TnT on the Cobas e801 analyzer (Roche Diagnostics) using the 9-min protocol. For internal QC we use the 2 Roche controls supplied as well as the BioRad control (mean hsTnT 16.5 ng/L). Consecutive patients with hs-TnT results below 12ng/L were re-tested in the same run using the 18-min protocol but these results were not released. Statistical analysis was performed using MedCalc software (MedCalc v19.1.6, Ostend, Belgium). Our laboratory is a College of American Pathologists (CAP) accredited laboratory and our performance on the CAP troponin external QA program is satisfactory. As this work was part of routine evaluation of laboratory assays, it was exempted from institutional board review.

Results: We studied 1731 men (age:15-101y, mean+SD: 54.52+16.79) & 1258 women (age:16-99y, mean+SD: 58.96+18.22). We divided the results into 2 hsTnT catego-

ries - below 5ng/L and 5-11.9ng/L and calculated the difference between the 9-min and 18-min results (Table 1). For hsTnT results below 5 ng/L, the difference between paired testing of hsTnT was below 2ng/L in 99.1% of the cases while it was less than 3ng/L in 99.5% of the samples with hs-TnT from 5-11.9ng/L.

Conclusion: Paired testing on the same samples show very close agreement. Thus we can be confident of the hsTnT results at the rule-out decision cut-points as recommended by the ESC for NSTEMI.

Table 1. Difference in hsTnT values between paired 9-minute & 18-minute assays

hsTnT categories	Difference between 9 min & 18 min hsTnT results				Total n
	< 1.0 ng/L n (%)	1.0-1.9 ng/L n (%)	2.0 – 2.9 ng/L n (%)	>3.0 ng/L n (%)	
< 5 ng/L	1262 (94.11)	67 (5.0)	8 (0.6)	4 (0.3)	1341
5 – 11.9 ng/L	1439 (87.3)	181 (11.0)	20 (1.21)	9 (0.55)	1649
< 12 ng/L	2701 (90.33)	248 (8.29)	28 (0.94)	13 (0.43)	2990

B-177

Relationship of Serum Ferritin with Extended Lipid Profile in Male AMI Patients

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Background: Serum ferritin concentration is considered to be the best clinical measure of body iron stores. Some studies have been in favor of ferritin being a risk factor for AMI. However,the association of serum ferritin with established risk factors for AMI like dyslipidemia has not been established unambiguously.**Objective:** To study association between serum ferritin levels and extended lipid profile parameters in AMI male patients. **Methods and Materials**: This case control study enrolled 40 male AMI patients (40-60 years) with equal number of age matched healthy (no history of angina/MI) individual. Confirmation of diagnosis of AMI was based on WHO criteria of clinical history suggestive of myocardial ischemia, ECG findings and raised biochemical markers like CK-MB and hs-Troponin T. They had not taken B vitamins and iron supplements for last 5 months(self-administered questionnaire); had no history of smoking, hematological disorders (anemia, polycythemia, myeloproliferative disorders), severe bleeding that required a blood transfusion, malabsorption syndrome, liver disease, kidney disease, diabetes mellitus, and hypertension.Serum ferritin was measured by electro-chemiluminescence immunometric method by Roche Cobas e411 analyzer. Serum iron, TIBC (total iron binding capacity), and extended lipid profile consisting of cholesterol (total, LDL, HDL),triglycerides were estimated by photometric and Lp(a), apolipoproteins, A1 and B, by immuno-turbidimetric methods on Roche Cobas c501 analyzer using commercially available kits from Roche.

Statistical Analysis: Unpaired student's t test was applied for statistical analysis. Normally distributed data is displayed as mean and 95% confidence interval (CI). Logistic regression was used to assess association of serum ferritin with extended lipid profile parameters. The p value of <0.05 was considered significant. Receiver operating characteristic (ROC) curve analysis was done to determine the predictive value of serum ferritin for AMI.

Results: Our study showed that serum ferritin levels in AMI patients and control group were 233.7 ± 39.8 ng/ml and 148.6 ± 33.2 ng/ml respectively (p<0.001). Patients with AMI had significantly high total cholesterol (228±27.3 vs 142±22.4mg/dl), LDL cholesterol(155±24.6 vs 86±14.2 mg/dl), triglyceride (196±32.3 vs 126±21.3 mg/dl), apolipoprotein B (158±18.6 vs 102±16.6 mg/dl) and Lp(a) (58±20.1 vs 26.1±12.4 mg/dl) (p<0.001) and significantly low HDL cholesterol(24±7.6 vs 44±9.6 mg/dl) and apolipoprotein A1(94±11.1 vs 132±16.2 mg/dl) levels than healthy subjects (p<0.001). Elevated ferritin concentrations were associated with significantly higher values for serum triglycerides, Total Cholesterol, LDL cholesterol, apolipoprotein B and lipoprotein(a) (p < 0.01), and lower levels for HDL cholesterol and apolipoprotein A-I (p < 0.05),in AMI patients. In ROC curve of serum ferritin level, the area under curve (AUC) was found to be 0.943 (95% confidence interval l=0.911 to 0.975). The optimal cut off value of serum ferritin level for predicting AMI was 198 ng/ml (sensitivity 88 % and specificity 82%).

Conclusion: This study showed that increased serum ferritin levels in conjunction with deranged extended profile could precipitate acute myocardial infarction in Indian male population. In addition, serum ferritin cut off value of 198 ng/ml had higher risk of AMI. These results suggest that measurement of extended lipid profile and ferritin could be of great assistance in predicating acute myocardial infarction.

B-178

Validation of the Lipid Profile on the Atellica Analyzer: New Quality Control Methodologies Implementation and Performance Evaluation of the Assays

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Background: The reduction of malnutrition and the increase in overweight and obesity in all age groups favored the appearance of chronic non-communicable diseases (CNCD), especially cardiovascular diseases. Dyslipidemia is one of these diseases and one of the main risk factors for atherosclerotic cardiovascular diseases. Fundamentally, they are diagnosed and phenotyped by laboratory determinations, therefore, the concern of all clinical laboratories is to release results with the maximum possible accuracy considering the best quality control management tools and performance of adopted methods. Sigma metrics have proven to be a powerful tool for objective and quantitative performance evaluation of tests and instruments. The objective of this study was to use sigma metrics to validate the lipid profile assays on the Atellica CH Siemens Healthineers platform in a large laboratory operating in Brazil and to create a scientific basis por planning the appropriate quality control strategy to assess these parameters performance. **Methods:** For the Cholesterol (Chol), HDL, LDL and Triglycerides (Trig) assays on the Atellica CH 1600 Analyzer, the precision and bias estimation peer group evaluation was performed by the repeatability study (%CV_R) and within-laboratory precision (%CV_{WL}), with 25 replicates total per QC sample for each assay. Method comparison studies were performed with the Atellica CH and AU 5800 Beckman Coulter assays according to EP09, using 50 serum samples that covered the entire linearity range. For the evaluation of sigma metrics, different goals of the total allowable error (TEa) were used, such as Ricos, RiliBÄEK, Biological Variation 2014 (VB2014), EFLM and CLIA. **Results**: The precision results are in agreement with the analytical quality specifications, with CV_{WL} of 1.9% to 1.4% and CV_R of 0.5% to 2.1%. For Chol and Trig, which have specifications in all the references mentioned, all results were greater than 6 sigma. Using the analytical quality specification of Biological Variation Ricos, an average sigma of 9.73 for Chol and 16.22 for Trig was observed, while using EFLM and CLIA, the mean sigma was 10.06 and 10.84 for Chol and 17.56 and 15.55 for Trig, respectively, all 6 sigma. Depending on the assay, the choice of TEa specification generates different results, as in the case of cholesterol with an average sigma of 10.84 using CLIA, and 14.17 using RiliBÄEK. The LDL and HDL assays were evaluated using the BV2014, one of the only references with data for both lipoproteins. All results obtained sigma above 3, ranging from 3.70 to 5.81 (good). **Conclusio**n: The tests demonstrated acceptable results of precision and sigma metrics. There is still no consensus on the appropriate source of TEa, which makes any sigma result complex to interpret. Thus, it is important to know the performance of each test to apply the most appropriate reference. Once the analyzer is running the tests routinely, these metrics will be evaluated periodically, observing the use of more realistic criteria to achieve the best performance the Atellica CH assays can offer. **Siemens Healthineers supported the studies by providing systems, and reagents.*

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Analytical Evaluation of the High Sensitivity Troponin Assay on Two Different Platforms in a Large Diagnostic Center, The Challenge of Interpretation

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Introduction: Patients who present symptoms suggestive of acute myocardial infarction (AMI) represent about 5 to 10% of the Emergency Services (ES) in Europe and in the United States[2]. In Brazil, coronary diseases are identified as the main cause of death, responsible for more than 30% of registered deaths[10]. The rapid identification of AMI is critical for the initiation of an effective medical treatment, in this context Troponin (cTn) plays an important role[3]. Siemens Healthineers has High Sensitivity Troponin assays (hs-cTnI) commercially available on different analytical platforms. The objective of this study was to evaluate the analytical performance of the hs-cTnI Dimension EXL compared to hs-cTnI Atellica IM. **Method:** hs-cTnI Atellica IM is a double capture sandwich immunoassay, uses three monoclonal antibodies[5]. The hs-cTnI Dimension ExL assay uses three monoclonal antibodies, LOCI technology to generate a signal. Quantification Limit Verification Study EP17-A26 was performed. Verification of the Imprecision studies were performed according to the CLSI EP15-A3 protocols[1], using a serum sample and three levels of quality control (QC). A linearity study was conducted considering the amplitude of the curve, with five points, according to EP6-A[7]. A comparison study between hs-cTnI Atellica IM and

hs-cTnI Dimension ExL was performed, at least, thirty-eight samples in each analyzer module. **Results:** The limits of quantification tested for Atellica IM and Dimension ExL were 3.58 ng/L and 6.00 ng/L respectively, CV of 9.25% and 10%. The linearity study (4.5 to 22636 ng/L) for Dimension ExL and Atellica IM presented R2 0.987 and 0.993, average CV 2.22% and 2.88% and average recovery of 15% and 9.63% respectively. For the imprecision study hs-cTnI Atellica IM with concentrations of 5.95 ng/L, 37.82 ng/L, 4058 ng/L and 11671 ng/L the CVs were 6.69%, 2.98%, 2.4% and 1.87% respectively; and for hs-cTnI Dimension ExL the concentrations were 6.38 ng/L, 43.9 ng/L, 4328 ng/L and 13143 ng/L and CV 8.29%, 3.56%, 3.01% and 5.42% respectively, the two analyzers presented less variation than suggested by the manufacturer. When comparing both hs-cTnI Dimension ExL and hs-cTnI Atellica IM assays, R2 = 0.9821 and Y=1.003*x+28.91 were obtained; no significant difference was observed in results below 60 ng/L (Dimension ExL = 14.11ng/L and Atellica IM = 12.59 ng/L, mean difference = -1.553 and SD = 4.76). **Conclusion:** The hs-cTnI Dimension ExL and Atellica IM assays have analytical performance as recommended by the new guidelines [9]. It is important to note that these tests demonstrate excellent precision below 7ng/L (CV <10%) and, to the 99th percentile, CV <5%, better than recommended of precision objectives in these important concentration ranges [2,4,5]. The hs-cTnI Dimension ExL assay produces higher concentrations than the Hs-cTnI Atellica IM, especially at lower concentrations. This difference is important for laboratory professionals and physicians to understand that different and specific cutoff points will be needed to optimize initial decision-making algorithms for AMI when tested on different platforms. Different methods within the same diagnostic center should be avoided for cTn. hs-cTn was designed to meet the guidelines regarding the serial measurement for the early diagnosis of AMI.**Siemens Healthineers supported the studies*

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Enhanced Akt3 Kinase Activity in Mice Reduces Atherosclerosis in a Gender Dependent Manner

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Background: Gender differences have been observed in atherosclerosis the susceptibility of atherosclerosis in many animal models. Akt3, one member of the serine/threonine kinase Akt/protein kinase B family, was demonstrated a macrophage-dependent atheroprotective function in mice. But However, whether the protective effect of Akt3 is in a gender dependent manner remains unknown. Macrophages, the major immune cell population in atherosclerotic plaques, contribute to the inflammatory response through foam cell formation and release of pro-inflammatory mediators, such as cytokines and chemokines. It was has been shown that Akt3 deficiency in macrophages promotes foam cell formation and atherosclerosis. , but Wwhether Akt3 affects pro-inflammatory cytokines release from macrophages is unclear. The purpose of this study is to determine the gender dependent role of Akt3 in atherosclerosis; , evaluate the effects of Akt3 on pro-inflammatory cytokines release from macrophages; , and to identify the possible mechanism.

Methods: Akt3^{nmf350} mice, A a mouse strain which has a missense mutation, (D229V), in the Akt3 gene that results in enhanced Akt3 kinase activity, were used in this study to assess the atheroprotective function of Akt3. We established the mouse model of ApoE$^{-/-}$/Akt3^{nmf350} mice, using ApoE$^{-/-}$ mice as control group, feed with western diet for 15 weeks. We fed male and female ApoE$^{-/-}$/Akt3^{nmf350} and control ApoE$^{-/-}$ mice with westernn type diet for 15 weeks andThen we measured the atherosclerotic lesion areas in of the entire aorta and the aortic root by Oil Red O staining. Analyzed the atherosclerotic lesion formation in male and female mice. To evaluate the effects of bone marrow derived macrophages on atherosclerosis, we created ApoE$^{-/-}$ chimeric mice with either ApoE$^{-/-}$/Akt3^{nmf350} bone marrow or ApoE$^{-/-}$ bone marrow. After 12 weeks of western diet, the atherosclerotic lesion areas in of the entire aorta and the aortic root were analyzed by Oil Red O staining in male and female mice. For selective cytokines detection, resident peritoneal macrophages from C57BL/6J and Akt3^{nmf350} mice were cultured overnight, then and cytokine concentrations were detected from cell media supernatant withvia Meso Scale Diagnostics's a custom 11-cytokines mouse U-PLEX panel and U-PLEX TGF-β Combo Mouse panelU-Plex assay platform. IL-6, MCP-1, TNF-α, TGF- β 2, MIP-1α, MIP-1β, IP10, IL-1β, IL-10 were analyzed. **Results:** Our data showed that enhanced Akt3 kinase activity in mice significantly reduced atherosclerosis, and the reduction in atherosclerotic lesion formation is was more pronounced in male mice. Reduced atherosclerosis was also observed in male ApoE$^{-/-}$ chimeric mice with ApoE$^{-/-}$/Akt3^{nmf350} bone marrow, but not in female mice. IL-6, MCP-1, TNF- α, and MIP-1α protein levels in the cell culture media of resident peritoneal macrophages from Akt3^{nmf350} male mice were significantly reduced compared to C57BL/6J male mice, but again there are were no differences in female mice.

Conclusions: These results demonstrated that the atheroprotective role of Akt3 is in a gender dependent manner and it these findings could contribute to improved individualized medicine.

B-183

A Novel Research-Use-Only Secretoneurin ELISA Assay Provides Superior Diagnostic and Prognostic Performance in Patients with Suspected Acute Coronary Syndrome Compared to the Established Radioimmunoassay

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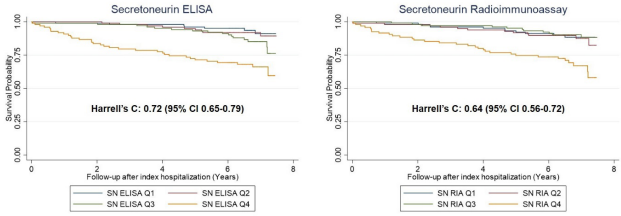
Background*:* Circulating Secretoneurin (SN) concentrations provide prognostic information in patients with cardiovascular disease. The performance of a recently developed research-use-only SN ELISA compared to the established SN RIA in patients with suspected acute coronary syndrome (ACS) is not known. **Methods***:* We included 401 unselected patients hospitalized with chest pain at a teaching hospital and adjudicated all hospitalizations as ACS or non-ACS. Blood samples were drawn <24 h from admission. The SN ELISA assay has analytical range 10-250 pmol/L, between run imprecision (n=20) of 5.5%, CV 4.1% at 23.7 pmol/L and 168.6 pmol/L. All-cause mortality was the primary outcome with mean follow-up 6.2 y. **Results***:* SN measured by ELISA and RIA correlated: rho=0.39, p<0.001. SN concentrations were higher in patients with ACS (n=161 [40%]) vs. non-ACS chest pain patients (n=240): SN ELISA median 32.8 (IQR 27.5-42.8) vs. 28.0 (24.5-34.0) pmol/L, p<0.001 and SN RIA 134 (115-154) vs. 123 (106-143) pmol/L, P=0.001. The C-statistics was 0.66 (95% CI 0.61-0.71) for the SN ELISA assay and 0.59 (0.54-0.65) for the SN RIA assay to separate chest pain patients with ACS from non-ACS chest pain patients. Sixty-five (16%) patients died during follow-up. Stratifying patients by quartiles of SN ELISA and RIA concentrations separated patients with poor and favorable prognosis (**Figure**). Adjusting for age, sex, blood pressure, previous myocardial infarction, atrial fibrillation, and heart failure in Cox regression, SN concentrations as measured by the ELISA, but not the RIA, remained associated with mortality: HR 1.71 (1.03-2.84), p=0.038. The C-statistics of the SN ELISA assay to separate patients with a poor and favorable prognosis was 0.72 (0.65-0.79) vs. 0.64 (0.56-0.72) for the SN RIA assay, p=0.007 for difference between the assays. **Conclusions***:* The novel SN research-use-only ELISA provides superior diagnostic and prognostic properties compared to the established SN RIA in chest pain patients with suspected ACS.



B-187

Use of a Novel High-Sensitivity Cardiac Troponin I Assay in a 0/1-Hour Algorithm to Rule-Out Acute Myocardial Infarction

B. Cook, J. McCord, M. Hudson, M. Moyer, A. Hana, G. Jacobsen, R. Nowak. *Henry Ford Hospital, Detroit, MI*

Background: High-sensitivity cardiac troponin (hs-cTn) assays can be used to rapidly rule-out acute myocardial infarction (AMI) in the Emergency Department (ED). Hs-cTnI 0/1-hour algorithms to rule-out AMI have been validated in non-United States (US) and recently, in US studies. Our objective was to a evaluate a novel hs-cTnI assay with a 0/1-hour AMI rule-out algorithm in patients presenting to the ED with symptoms suspicious for AMI. Additionally, the 30-day prognostic utility of hs-cTnI in patients ruled out for AMI with this 0/1-hour algorithm was assessed.

Methods: Patients presenting to the Henry Ford Hospital (HFH) ED (Detroit, Michigan) with any symptoms suspicious for AMI and had a troponin measurement ordered by the ED physician were enrolled. There were no patient exclusion criteria. Study blood specimens were collected at baseline and at 1 hour, processed and stored at -80 degrees C within 1 hour. Hs-cTnI was measured in the HFH clinical laboratory using the Access hs-cTnI assay (overall 99th percentile upper reference limit (URL) of 17.5

ng/L; Access 2, Beckman Coulter, Brea, California). The diagnosis of Type 1 or Type 2 AMI was adjudicated by two cardiologists using the Third Universal Definition of MI and the Elecsys Troponin T Generation 5 assay (overall URL 14 ng/L; Cobas e601, Roche Diagnostics, Indianapolis, IN), not contemporary troponin values, and using all available clinical data 30 days after presentation. The adjudicating physicians had access to 0-, 1- and 3-hour hs-cTnT values that were obtained during a previous clinical study. The 0/1-hour hs-cTnI rule-out algorithm evaluated in our report consisted of a 0-hour (presentation) value of <4 ng/L or a 0-hour of <5 ng/L and a 1-hour delta value of <4 ng/L.

Results: Of 552 enrolled patients 45 (8.2%) had an adjudicated AMI (27 Type 1; 18 Type 2). At 30 days, AMI/death occurred in 11 (2.0%) patients (3 cardiac deaths, 8 additional AMIs) with 2 non-cardiac deaths. Of the 270 (48.9%) patients categorized to 0/1-hour rule-out one patient had an adjudicated AMI, (negative predictive value 99.6%, confidence interval (CI) 98.0% to 100.0%; sensitivity 97.8%, CI 88.2% to 99.9%), comparing favorably with the findings of a recent multicenter US study by Nowak, who reported a NPV of 99.7%, CI 99.0% to 100.0%, and a sensitivity of 99.1%, CI 96.9% to 99.9% using the Siemens hs-cTnI assay for a 0/1 hour rapid ED evaluation for AMI. In our trial there were no cardiac deaths and no AMIs at 30 days, with 1 non-cardiac death.

Conclusion: In this single center US study at an urban ED, a 0/1-hour algorithm using a novel hs-cTnI assay, not previously studied in the US, effectively ruled out AMI in an all-comer's cohort of ED patients, when AMI was adjudicated using hs-cTnT values. Additionally, these ruled-out AMI patients had very low 30-day AMI/death rates. We obtained clinically acceptable high NPVs but a sensitivity of less than the desirable 99%. Further multicenter studies completed in the US are needed to confirm our results and improve the sensitivities of this rapid rule-out algorithm.

B-188

Development of Liquid-Stable Calibration Verification Sets for Human Cardiac Markers to Characterize Method Linearity and to Verify the Reportable Ranges

C. Happe, J. Herod, J. Pawlak, M. Sweatt. *LGC Maine Standards, Cumberland Foreside, ME*

Introduction: There are a number of cardiac markers available for testing on automated chemistry analyzers. These analytes fall under two categories: (1) cardiac biomarkers, which can indicate a cardiac-related event, such as a heart attack, and (2) markers for cardiovascular disease (CVD) risk, which are used to evaluate the risk for future cardiac-related events. Creatinine Kinase-MB (CK-MB) and myoglobin (MYO) are cardiac biomarkers released at elevated levels due to muscle damage or injury, particularly during a cardiac event. Troponin I (TnI) and troponin T (TnT) are cardiac-specific proteins that are released within a few hours of heart damage; high-sensitivity (hs) methods for both allow for earlier detection. B-type natriuretic peptide (BNP) and N-terminal pro BNP (NT-proBNP) are two different proteins that are both released in response to congestive heart failure (CHF). Some cardiac biomarkers can also be used as markers for CVD risk. hsTnI and hsTnT detect low troponin levels which can indicate an increased risk of future cardiovascular events. BNP and NT-proBNP can be used to monitor CHF. Traditional markers for CVD risk include total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and high-sensitivity C-reactive protein (hsCRP). Elevated levels of CRP can indicate inflammation or infection, however, consistent low levels of CRP (measured using hsCRP) can be associated with atherosclerosis and used to evaluate CVD risk. Additional CVD risk factors include apolipoproteins A and B, homocysteine and lipoprotein (a) (Lp(a)). Elevated levels of homocysteine can indicate increased risk for blood clot formation, heart attack, or stroke. These analytes are among the most commonly tested cardiac markers for both cardiac-related events and CVD risk. LGC's objective was to offer an expansive product line containing these analytes in Cardiac Marker 1 (401), Cardiac Marker 2 (402), High Sensitive Troponin (405RO) and Cardiac Marker 3 (409RO). LGC also offers a Lipoprotein line including Lipoprotein 1 (501) and Lipoprotein 2 (507RO). Methods: Each product in the VALIDATE® Cardiac Marker and Lipoprotein lines is formulated as a single or multi-constituent kit, in a human serum matrix, according to CLSI EP06-A into five equal-delta concentrations to cover the reportable ranges of each analyte on the respective platforms. For each level, samples were tested in triplicate for each of the analytes. Reported recoveries were evaluated for mean, SD, and linearity using MS-DRx®, LGC's proprietary linearity software. Limits

05-200996-00 Development of Cardiac Marker Cal Ver Abstractwere applied as a percentage of the total allowable error (TE_a), specific for each analyte. Product stability was determined by a combination of stress and real-time stabilities. Results: For the VALIDATE® Cardiac Marker and Lipoprotein lines, all levels are stable for their claimed expiration and evaluate the assay's linearity through the manufacturers' reportable range. Conclusion: These currently available Calibration Verification test kits are listed with the FDA, CE marked and aid in testing and documenting of calibration verification, analytical measurement range verification and linearity which are required by many inspection agencies. The products also support the user when troubleshooting instrument systems, reagent problems and calibration anomalies.

B-189

Clinical Impact of Implementing the Novel NIH Equation to Calculate LDL-C in Hypertriglyceridemic Populations

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Background: Low-density lipoprotein cholesterol (LDL-C) is a key target in the clinical evaluation and management of cardiovascular disease. Most clinical laboratories estimate LDL-C using total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) in the Friedewald equation ($LDL\text{-}C_F$ = TC - HDL-C - [TG/5]). Friedewald estimated $LDL\text{-}C_F$ has reduced accuracy in hypertriglyceridemic individuals. Homogeneous direct assays ($LDL\text{-}C_D$) are commonly used when TG >400 mg/dL (4.5 mmol/L). Two new estimates have recently been developed with reportedly superior accuracy in hypertriglyceridemia. The Martin-Hopkins equation ($LDL\text{-}C_{MH}$=TC - HDL-C - [TG/X]) uses an adjustable variable empirically defined from the vertical auto-profile (VAP test) method. The NIH equation ($LDL\text{-}C_{NIH}$ = [TC/0.948] - [HDL-C/0.971] - [TG/8.56] - [(TG × (TC-HDL-C)/2,140] + [TG^2/16,100] -9.44) was modeled based on the LDL-C reference method ß-quantification ($LDL\text{-}C_{BQ}$) in a population enriched with hypertriglyceridemia. Our objective was to determine the difference in reported LDL-C by various methods among subjects with hypertriglyceridemia.

Methods: Comparisons using the two new LDL-C estimates were made in different cohorts with TG 400-800 mg/dL. Cohort 1 consisted of patients with a gold standard $LDL\text{-}C_{BQ}$ (n=1,176); cohort 2 included basic lipid panels with TG >400mg/dL that reflexed to $LDL\text{-}C_D$ (n=12,971). Concordance between LDL-C methods was assessed by Spearman's ρ, mean absolute difference, percent of patients within 12% of the reference method, and classification at 70, 100, 130, 160 and 190 mg/dL LDL-C treatment thresholds.

Results: In cohort 1, the median (IQR) concentration for TG was 486 mg/dL (435-565), $LDL\text{-}C_{BQ}$ was 110 mg/dL (83-140), $LDL\text{-}C_{NIH}$ was 111 mg/dL (83-143), and $LDL\text{-}C_{MH}$ was 132 mg/dL (103-163). Correlation with $LDL\text{-}C_{BQ}$ was 0.904 in both estimates. Mean difference from $LDL\text{-}C_{BQ}$ was -0.6 mg/dL for $LDL\text{-}C_{NIH}$ compared to +19.8 mg/dL for $LDL\text{-}C_{MH}$ (P<0.01). Consequently, 57% of $LDL\text{-}C_{NIH}$ values were within 12% of $LDL\text{-}C_{BQ}$ compared to 32% for $LDL\text{-}C_{MH}$. $LDL\text{-}C_{NIH}$ classified 64% concordant (18% higher; 18% lower), while $LDL\text{-}C_{MH}$ was 43% concordant (54% higher; 2% lower). In cohort 2, the median (IQR) $LDL\text{-}C_D$ was 113 mg/dL (85-145) compared to 107 mg/dL (84-134) for $LDL\text{-}C_{NIH}$ and 118 mg/dL (IQR 95-145) for $LDL\text{-}C_{MH}$. The mean difference from $LDL\text{-}C_D$ was -7 mg/dL for $LDL\text{-}C_{NIH}$ and +5 mg/dL for $LDL\text{-}C_{MH}$. Concordance by $LDL\text{-}C_{NIH}$ was only 55% (33% lower; 13% higher) compared to 61% by $LDL\text{-}C_{MH}$ (13% lower; 26% higher). In a small subset of samples with both $LDL\text{-}C_{BQ}$ and $LDL\text{-}C_D$ measured (n=63), $LDL\text{-}C_D$ was within 12% of $LDL\text{-}C_{BQ}$ in 28 (44%) samples compared to 37 (59%) for $LDL\text{-}C_{NIH}$ and 20 (32%) for $LDL\text{-}C_{MH}$.

Conclusion: Both the NIH and Martin-Hopkins equations were strongly correlated with the β-quantification reference method, yet changed classification in more than a third of patients. $LDL\text{-}C_{MH}$ reclassified most patients into a higher risk category compared to $LDL\text{-}C_{BQ}$. When compared to $LDL\text{-}C_D$, $LDL\text{-}C_{MH}$ concordance was higher than $LDL\text{-}C_{NIH}$. The difference in performance may be due to bias of direct LDL-C, which has been reported to overestimate LDL-C when TG are elevated. The inconsistencies across methods provide caution when changing methods in serial measures.

B-190

Experiences Implementing Non-Fasting Lipids in a Large Academic Hospital

K. Morel, A. J. McShane. *Cleveland Clinic Foundation, Cleveland, OH*

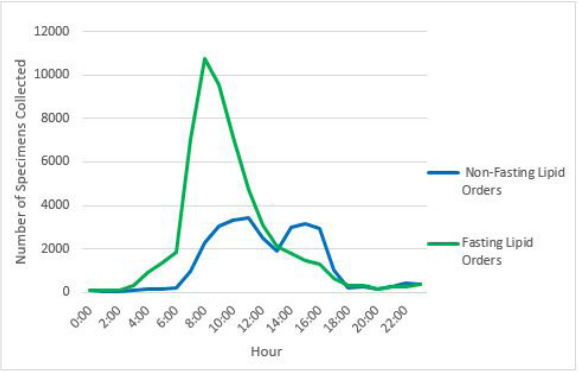
Background: The fasting lipid panel is a routine laboratory test utilized to assess risk of atherosclerotic cardiovascular disease (ASCVD), amongst other conditions. Historically, the specimens are collected after a 12-hour fast. Recent guidelines support the measurement of non-fasting or fasting lipids to assess initial risk in an untreated patient. For the patient, this eliminates potential barriers of fasting and returning for phlebotomy. The laboratory may benefit from a more steady patient flow in phlebotomy areas versus a morning bolus.

Methods: Data was collected from January 1, 2019 to December 31, 2019. Data sets include inpatient and outpatient samples. Both panels, fasting and non-fasting, were performed on a Roche cobas 8000 platform. Panels include the following components: Triglycerides, Total Cholesterol, HDL Cholesterol, Calculated VLDL Cholesterol, Calculated LDL Cholesterol, Calculated Total Cholesterol to HDL Ratio, Calculated LDL to HDL ratio, and Calculated Non-HDL Cholesterol.

Results: A total of 85,933 lipid panels were resulted of which 35% were non-fasting. The non-fasting LDL Cholesterol had an average of 98 mg/dL and a median of 95 mg/dL. The fasting LDL Cholesterol had an average of 98 mg/dL and median of 95 mg/dL. Of the 55,899 fasting specimens collected, only 708 (1%) reflexed a Direct LDL Cholesterol due to a Triglycerides value greater than 400 mg/dL. For the non-fasting LDL Cholesterol, 695 results (2%), suggested a fasting lipid be ordered due to the elevated triglycerides value. When plotted by hour of day, it was noted that 56% of non-fasting orders were collected between 1100 and 1700 (Figure-1).

Conclusion: Over a 1-year period, the means and medians for LDL Cholesterol were similar for the fasting and non-fasting lipid panels. The non-fasting lipid panel allowed for more draws between 1100 and 1700, alleviating some strain on phlebotomy centers during morning hours.

Figure 1: Non-Fasting versus Fasting Lipid Panel Specimen Collection over 24 hours

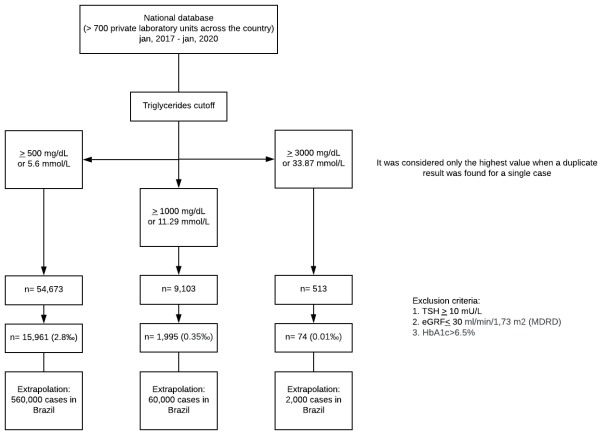


B-191

Finding Patients with Severe Hypertriglyceridemia through Bigdata Analysis in the Brazilian Population

M. H. C. G. CASTELO[1], C. M. M. PONTE[2], L. A. A. BATISTA[2], L. C. VIEIRA[3], R. F. B. CANINEU[3], F. P. OLIVEIRA[3], L. L. A. CAVALCANTE[1], S. P. BANDEIRA[1], T. P. ALMEIDA[2], G. A. CAMPANA[3]. *[1]DASA, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]DASA, SAO PAULO, Brazil*

Background: Very high triglycerides (TG) is characterized by a fasting TG > 500 mg/dL. Several clinical conditions are associated with this finding, including rare genetic causes. Identifying such patients has been a challenge in daily practice, and probably many cases of primary hypertriglyceridemia can be diagnosed. **Objective:** The aim of this study was to propose an identification flow of patients with very high TG from a large national database of laboratory tests. **Methods:** This is a cross-sectional study to evaluate all patients with TG analyzed in the national laboratory bigdata in Brazil, from January 2017 to January 2020. The TG results were divided into 3 categories (> = 500mg/dL, 1000mg/dL, and 3000 mg/dL) and distributed by sex and age group. Subsequently, filters were applied to exclude patients with secondary causes that can lead to high TG: TSH>=10.0 mUI/mL, eGFR<= 30 ml/min and A1c> = 6.5%. **Results:** TG measurements of 5,678,569 patients were performed. Of these, 54,673 (9.6%) had TG> = 500 mg / dL; 9.103 (1.6%) TG> = 1000 mg / dL; and 513 (0.09%) TG> = 3000 mg / dL. The distribution by sex and age showed no difference between groups. After excluding patients with hypothyroidism, decompensated diabetes and renal failure, the prevalence of TG> = 500 mg/dL was 2.8%; TG>= 1000 mg/dL was 0.35%; and TG>= 3000 mg / dL was 0.01%. Considering that the brazilian population is approximately 200 million inhabitants, we estimate that about 560,000 people in Brazil have TG> = 500 mg / dL; 60,000 TG>= 1000 mg / dL; and 2,000 TG>= 3000 mg / dL. (figure1). **Conclusion:** The use of a national bigdata seems to be important for knowledge of prevalence estimate of rare diseases such as severe hypertriglyceridemia. The definition of cutoff points to exclude secondary causes are essential in this process.



B-193

Initial Evaluation of Abbott ARCHITECT High Sensitivity Troponin-I Assay and Comparison of EDTA and Heparin Plasma Sample Types

K. Lao, N. A. Hordynsky, T. Swift, Q. Sun, S. M. Truscott. *Beaumont Health, Royal Oak, MI*

Background: High sensitivity assays for troponin have recently been approved for clinical use in the United States. These assays have the potential to enhance the management of emergency center cases of acute chest pain and improve the detection of acute coronary syndromes, especially in women. Implementation of these assays is a careful exercise that laboratories are undertaking in stages. Working with emergency and cardiology physicians, laboratories can facilitate test ordering and workflows that maximize the benefits of the high sensitivity assays and minimize disruptions to resource allocation. One of the first steps is validation of manufacturer claims for method performance characteristics; these data assist in the determination of appropriate cutoffs and triage procedures. Our initial study compared the Abbott ARCHITECT High Sensitivity Troponin-I (hsTnI) assay to the contemporary Abbott TnI II assay on the i2000 instrument. We also compared EDTA and heparinized plasma samples drawn at same time.

Methods: The Abbott hsTnI assay is a paramagnetic microparticle-based acridinium chemiluminescent immunoassay. Method comparison between the two assays was performed using EDTA samples (n = 77) collected over the prior 24 hours and stored at 4°C. Linearity was verified by serial dilution, and within-run and between-run (10-day) imprecision was evaluated near the claimed limit of quantitation (LoQ). Furthermore, comparison of two tube types (n = 28 paired EDTA and lithium heparin gel separator) was performed from remnant patient samples drawn simultaneously. All studies were performed using a single lot of reagent.

Results: Comparison of EDTA samples between hsTnI and contemporary assays spanned the range of 6.1 to 3406.6 ng/L, and Passing-Bablok regression found a slope of 1.076 (confidence interval, CI: 1.016 - 1.145) and intercept of -1.0 (CI: -3.0 - 1.4). The approved measuring range of 3.5 - 5000.0 ng/L was verified using samples with assigned values in the range 5.8 - 4648.0 ng/L; slope and intercept were 0.991 and 0.4 respectively. Total imprecision was 11.1 %CV at 4.1 ng/L, which is near the claimed LoQ of 3.5 ng/L. Nearer to the upper reference limits, the imprecision was 5.6% (at a mean of 20.8 ng/L). EDTA and lithium heparin tube types correlated with a slope and intercept of 1.068 (CI: 1.025 - 1.128) and intercept of 2.0 (CI: -2.3 - 5.4).

Conclusion: The Abbott hsTnI assay meets precision claims and correlates adequately with the prior generation troponin assay. With fresh samples, the comparison between EDTA and heparinized plasma samples was acceptable. Future studies will include validation of sex-specific upper reference limits, verification of manufacturer claims for limit of quantitation, and consistency of method performance characteristics across lots of reagents. All of these studies will be crucial to informing conversations with cardiology and emergency center physician colleagues as the test is implemented.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Proteomics & Metabolomics

B-197

Unraveling the Androgen Receptor Variants Protein Expression Landscape using Targeted MS

Z. E. Sychev, J. Drake, S. Dehm. *University of Minnesota, Minneapolis, MN*

Background: Castration Resistant Prostate Cancer (CRPC) is a treatment resistant form of prostate cancer (PCa) with no cure. Currently, there is no way to identify which patients will develop this resistance until full blown CRPC develops. Therefore, all PCa is treated the same resulting in tumor regression in some cases and progression in others. Targeting the androgen receptor (AR) is still the focus of current therapies even in CRPC. Emergence of AR splice variants (ARVs) after initial treatment is thought to be one of the primary mechanism of resistance. ARVs lack the ligand binding domain rendering Androgen Deprivation Therapy (ADT) ineffective in tumors expressing these variants. Recent work has identified the DNA and RNA species of ARVs in CRPC but investigation into whether the protein is translated and functional are unknown. One exception is the approval of an antibody test that detects a specific ARV, ARv7, from the blood of PC patients and predicts ADT response. However, there are instances where a patient may not express ARv7 and are still resistant to ADT. This may suggest that other ARVs, not currently detected at the protein level, are important predictors for ADT response. **Methods:** We have developed a targeted mass spectrometry-based (MS) proteomics assay that evaluates protein identification of known and unknown ARVs that may predict response to ADT. Targeted MS provides accurate, precise, and reproducible detection of a pre-determined set of peptides from multiple samples with high sensitivity without the need for antibody enrichment. We used an mRNA guided approach and generated a library of peptides that is used to calibrate the mass spectrometer and identified each ARV uniquely and reproducible. **Results:** Using this approach, we have identified two known ARVs, which validates our assay, and four novel ARVs via the mRNA-guided approach in models of PCa cell lines and tumors. **Conclusion:** This proposal provides a platform to set up a system for future functional studies in pre-clinical models and then ultimately clinically evaluate endogenous ARVs levels that may provide clues to resistance mechanisms to therapies and as biomarkers for patient stratification leading to treatment decisions. The outcomes of these studies will establish a prognostic biomarker program that measures ARVs proteins in real time from clinical biopsy tissues, circulating tumor cells, or exosomes and informs the clinician on whether ADT may be effective for each PCa patient.

B-198

Development of Urinary Exosome Proteins and Phosphoproteins as Non-Invasive Diagnostic Markers for Parkinson's Disease

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Background: Extracellular vesicles (EVs), exosomes, have emerged as a rich resource for the discovery of disease-relevant biomarkers from biofluids. These EV-based markers can be identified well before the onset of symptoms or physiological detection of disease, making them promising candidates for early-stage diagnostic applications. Moreover, EVs are a promising source of potential phosphoprotein-based biomarkers, considering that many phosphorylation events directly reflect cellular physiological status. Here we present our development efforts of proteins and phosphoproteins from extracellular vesicles in urine as biosignatures for Parkinson's disease diagnostics. Parkinson's disease (PD) is the second most common neurodegenerative disorder among the elderly population. Within the disorder, LRRK2 gene mutations have been recognized as genetic risk factors for both sporadic and familial forms of PD. LRRK2 is known to phosphorylate a subgroup of Rab proteins and regulate their ability to bind the effector proteins. Rab proteins are now well-recognized as the regulators of vesicle formation and exosome secretion.

Methods: In this study, we utilized a total of 82 PD-relevant samples available through the LRRK2 Cohort Consortium with and without the common Gly^{2019} →Ser (G2019S) mutation. We focused on LRRK2 and its substrates because this sample cohort has been uniquely curated for in-depth analysis and comparison of LRRK2 genotype and activity effects on PD. We utilized our unique combination of in-house developed EVtrap exosome isolation technology and LCMS-based phosphoproteomics approach for effective EV extraction and in-depth proteomic biomarker discovery from urine. After isolation of EVs from urine samples of PD patients and healthy individuals, we extracted and digested the exosomal proteins. The majority of each sample was used for phosphopeptide enrichment, while ~2% of each was utilized for direct proteomics analysis (resulting in total of 164 LC-MS runs).

Results: In total, we identified and quantified over 2,700 unique phosphoproteins and over 4,500 unique proteins from the urine EV samples. Relative quantitation revealed interesting and potentially actionable patterns for stratification of PD-/+ samples, as well as the signaling influences of the LRRK2 G2019S mutation. We further analyzed the LRRK2 effects on urinary exosomes by Western Blot analysis of exosome marker in comparison to LRRK2 phosphorylation.

Conclusion: These efforts resulted in identification of several dozen novel PD-relevant phosphoprotein markers in addition to identifying phosphorylation events in the LRRK2/Rab network. Our method to date is the first to successfully demonstrate the feasibility of developing biofluid-derived EV phosphoproteins for disease profiling.

B-199

Development and Validation of Label Free LC-MS/MS Protein Sulfhydration Profiling from Frozen Tissues for Potential Risk Assessment in Parkinson's Disease

N. K. Bithi[1], Y. Henderson[2], C. Link[2], B. Willard[3], C. Hine[2]. *[1]Cleveland Clinic Lerner research Institute/Cleveland State university, Cleveland, OH, [2]Cleveland Clinic Lerner research Institute, Cleveland, OH, [3]Proteomics and Metabolomics Core, Lerner research Institute, Cleveland, OH*

Objective: Endogenously produced hydrogen sulfide (H2S) serves as a beneficial bioactive metabolite, and lack thereof correlates to clinical conditions like hypertension, neurodegeneration, and diabetes. A mechanism of action for H2S is through modifications of cysteine residues known as protein sulfhydration. Examples of protein sulfhydration and its functional consequence include TRPV4 (transient receptor potential cation channel subfamily V member 4) which promotes vasodilation, and Parkin which increases its ubiquitin ligase activity contributing to neuroprotection. Uncovering multi-organ and/or blood-based sulfhydration profiles can provide valuable resources for biomarker studies. However, most sulfhydration studies were based on cell culture models or with purified proteins. The objectives of this study were to develop and validate a proteomics-based platform for the detection of sulfhydrated proteins in tissue and plasma under dietary restriction conditions known to modulate endogenous H2S production and then, identification of sulfhydrated protein which related to neurodegeneration. Methods: Using a modified biotin thiol assay (BTA), we isolated sulfhydrated proteins from 20-month old male mice fed ad libitum or fasted every-other day (EOD) for 2.5 months (n = 5/group). BTA was applied at pH 7.4-7.5 to flash frozen tissues via: 1) lysis with RIPA buffer, 2) protein concentration determination and normalization, 3) 7 mg of protein incubated with 343 μM Maleimide-PEG2-biotin, 4) precipitation of the alkylated protein with acetone, 5) incubation of precipitated proteins overnight in spin columns containing streptavidin-agarose resin, 6) multiple washes, and 7) elution with and without dithiothreitol (DTT). After BTA, 11 μL of eluate was run through 0.75 mm Sodium dodecyl sulfate (SDS)-12% polyacrylamide gels for ~15 minutes at 125 volts and visualized by staining. Protein bands were fragmented, reduced and alkylated with DTT and iodoacetamide, trypticly digested, and peptides analyzed on a Orbitrap Fusion Lumos Tribrid mass spectrometer. Bioinformatics analysis for identification and relative abundance via spectral counting of sulfhydrated proteins and their functional enrichment was by Mascot, SEQUEST, Scaffold, and KEGG software database tools. BTA was also validated via western blot analysis. Results: We identified 1158, 1211, 530, 446, 731 and 170 sulfhydrated proteins in late-age mice and their associated biological pathways in the liver, kidney, heart, muscle, brain and plasma, respectively. EOD fasting enriched the number of sulfhydrated proteins in liver, kidney, muscle, and brain while it decreased these in the heart and had minimal impact in plasma. Sulfhydrated proteins were involved in numerous biological and clinically relevant pathways, notably metabolic, protein homeostasis, and neurodegeneration. One striking finding was the enrichment for DJ-1 (Park7) sulfhydration in brain under EOD. Deficiency in Park7 activity is known to contribute to Parkinson's disease (PD) clinical symptoms, as PD patients with mutated Park7 exhibit early onset of hypokinesia and rigidity followed by anxiety and cognitive decline. We found that late-onset EOD fasting improved cognitive function, correlating ($P<0.02$) with enhanced Park7 sulfhydrated protein in brain. Conclusion:

These methods will be useful for potential gain and/or loss of protein sulfhydration profiling in clinical biomarker studies from biopsied tissues or serum as shown in this study for PD.

B-200

Using a Novel LC/MS/MS Method to Identify Acute Kidney Markers

L. Macnamara, B. Pang, M. Rocco, S. Wong. *Wake Forest University School of Medicine, Winston Salem, NC*

Background: Acute kidney injury (AKI) occurs when there is a sudden decrease in kidney function over a short period of time. The measurement of serum creatinine and/or urine output is typically used to identify AKI. It is difficult to diagnose AKI before a rise in creatinine or the precise level of kidney function during an AKI event. Targeted metabolomics may serve as supplemental biomarkers for more accurate prognosis. Using LC/MS/MS assays, other analytes in urine samples that are part of different biochemical pathways can be measured at the same time. In this study, metabolites found in urine are measured and compared to age and gender matched urine samples of individuals with no history of AKI. **Methods:** The novel method was developed on a research 6500 Triple Quadrapole mass spectrometer to measure the concentrations of creatinine, succinate, hippurate, sorbitol, citric acid, trimethylamine oxide (TMAO), glucose and oxoglutarate in urine samples. The method uses electrospray ionization in both a negative and positive ion mode. A Shimadzu Nexeral LC with a Phenomenex Synergi 4µm Fusion RP 80Å (100 x mm) separation column and a Phenomenex SecurityGuardTM guard cartridge kit with an analyte separation. With the exception of oxoglutarate, isotope labeled internal standards were used for each analyte. Isotope d4-succinate was used as the internal standard for oxoglutarate. Urine samples from adult patients with AKI and control samples from individuals without AKI were obtained to analyze changes in analyte concentration. Patients with AKI were consented and urine was collected every 48-72 hours and stored at -20°C. Analyte variations over time were evaluated to identify patterns in AKI patients. **Results:** Urinary metabolites of AKI patients may be measured using a rapid and accurate LC/MS/MS method. Inter-day precision ranged from 1-9.52% and intra-day precision ranged from 0.52-10.48%. Current results from de-identified adult urine samples show changes in the biomarkers being analyzed. Oxoglutarate, a master regulator of carbon and nitrogen pathways and acid-base balance, was found to have a 480-fold increase in one patient (RM003) over the course of one month while creatinine only had an 3.6-fold increase during that time period, suggesting that oxoglutarate may be an analyte of particular interest. Increases in succinate levels are linked with hypertension and preliminary results indicate that patients with high levels of creatinine also have an increase in succinate levels with RM003 having a 39-fold increase in succinate. An increase in TMAO is correlated with reduced renal function and cardiovascular disease. TMAO levels increase in patients with high creatinine levels. **Conclusions:** Changes in concentrations of these analytes of AKI patients may be helpful for earlier detection of AKI as well as possibly identifying patients at increased risk for developing AKI.

B-201

Evaluating Reference Methods and Materials for Vitamin D Binding Protein Quantification

L. Kilpatrick[1], A. Boggs[2], C. Davis[2], C. Henderson[3], A. Hoofnagle[3], S. Long[2], J. Yen[1], K. Phinney[1]. *[1]NIST, Gaithersburg, MD, [2]NIST, Charleston, SC, [3]University of Washington, Seattle, WA*

Background: Vitamin D binding protein (VDBP) is the primary transporter of vitamin D metabolites in serum to target tissues. VDBP concentrations change during pregnancy, trauma, sepsis and diseases associated with inflammation and may indicate patient outcomes. Additionally, the importance of unbound or bioavailable fractions of 25-hydroxyvitamin D in health is under investigation. Both areas of study require accurate quantification of VDBP. Recent studies show that immunoassays for VDBP may not give comparable results; therefore, a reference method and reference materials are required for standardization of results. In this work, a method using liquid chromatography-isotope dilution mass spectrometry (LC-IDMS) was evaluated and used to quantify VDBP in pooled plasma and serum and assign reference values.

Methods: VDBP concentrations were quantified in Standard Reference Materials® (SRMs, www.nist.gov/srm) 1950 Metabolites in Frozen Human Plasma and 1949 Frozen Human Prenatal Serum (a 4-level material of serum collected from women prior to pregnancy and during each trimester). SRM 1950 vials were prepared in two laboratories using similar methods. Two aliquots were removed from 14 vials. Samples were denatured with trifluoroethanol, reduced with TCEP, alkylated with iodoacetamide and digested with trypsin. Digested samples were analyzed with LC-IDMS using isotopically labeled peptides as internal standards. The experiment was repeated three times at each site. Protein concentrations were calculated from the average concentrations of selected peptides. SRM 1949 was analyzed by one laboratory using 3 vials, from each of the four levels, prepared in triplicate using the method described above.

Results: The VDBP concentration in SRM 1950 determined from data measured by two laboratories was 175 ± 18 mg/L. Data from each laboratory had < 4 % CV and < 6 % CV when comparing both laboratories. Quantification of VDBP in SRM 1950 gave consistent concentrations over 8 months (< 4 % CV). For SRM 1949, data from one laboratory had < 1.4 % CV for each level of the material and the VDBP concentration was found to increase from 212 ± 3 mg/L to 383 ± 5 mg/L as pregnancy progressed.

Conclusion: LC-IDMS analysis of VDBP in plasma and serum were found to give repeatable and reproducible results. SRM 1949 and SRM 1950 are now the first available reference materials for VDBP quantification.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Laboratory Management

B-203

Use of Electronic Text Processing for Automated, Paperless Review of Daily Quality Assurance Reports Generated by the Laboratory Information System

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Background: Review of laboratory results is conducted daily for error detection and correction in accordance with accreditation standards for quality assurance (QA). At our institution, the starting point of this process is a daily QA report generated by the hospital information system (EPIC). This report lists all results having critical values, delta values, linearity failure values, or non-numerical results. Manual review of this report is conducted daily to determine whether further action regarding any individual sample is required. Our objective in this study was to produce a computer program able to "read" the original QA report electronically rather than manually, and able to apply certain rules of review so as to produce a condensed report inclusive only of those QA report entries that require follow-up. **Methods**: The EPIC QA daily report was exported and saved as a comma-separated-variables file (*.csv). A text analyzer program (Quality Assurance Data Reduction, or QADR) was written in Python to "read" the *.csv file. QADR was programmed to exclude from further consideration those QA report entries that required no further action. This involved comparison of file contents across multiple fields for each entry, or across multiple entries for each sample. Rules implemented by programming were as follows: *A*. Entries with ">" results were excluded for those analytes for which ">" is appropriate (free T4, free T3, total T3, folate, HbA1c). *B*. Entries with "<" results, or with delta checks (for 12 analytes), were excluded unless the same sample exhibited more than one entry in either category across different analytes. *C*. Critical values entries were excluded if the comment field contained the appropriate "Read back and acknowledged" or "Call-back not required" canned comments. The output of QADR was a text file containing summary statistics of types of entries as well as a simple list of accession numbers for all samples that did not pass criteria for exclusion. **Results**: The QADR program is currently under a 30-day trial/validation period, consisting of side-by-side comparison of program output to results of manual review, in order to detect and correct any as-yet-unseen errors of omission. Based on trial period results thus far, electronic review by QADR results in a final written report that is greatly shortened compared to the length of the original QA report. Typically, a 50-page printed report containing some 600 entries is reduced to a three-page report, with elimination from further consideration of more than 90% of the original report entries. In comparison to manual review, time needed to identify samples requiring follow-up using QADR is reduced from approximately 30 min to 10 min. **Conclusions**: We developed a computer program in Python to electronically "read" and interpret our daily QA report, so as to streamline the process of identification of samples requiring follow-up review. Based on trial period results, it is estimated that deployment of the QADR program will produce a time savings of approximately 100 man-hours per year, and eliminate printing and storage of more than 12,000 pieces of paper per year.

B-204

Establishment and Implementation of an Improvement Plan using Lean Six Sigma Methodology to Minimize Variation in Lab Tests Ordering

O. Elgaddar, S. Gomaa, R. Rashwan, M. Abaza, N. Dowidar. *Medical Research Institute, Alexandria University, Alexandria, Egypt*

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. The present study aims at establishing and implementing an improvement plan using Lean Six Sigma methodology to minimize variation in the ordered lab requests in the Chemical Pathology Department of the Medical Research Institute - Alexandria University.

METHODS: Six Sigma approach methodology, namely, Define, Measure, Analyze, Improve and Control (DMAIC) phases was applied. The Define phase started by selecting Hepatology department (That sends 18.8% of the hospital lab requests), Stakeholders analysis was done, followed by Voice of Customer (VOC) which was converted to Critical to Quality (CTQ). The Measure phase included drawing a Flow Chart and Swim Lane diagram, then a Brainstorming session with stakeholders that was summarized in an affinity / Ishikawa (Fish bone) diagrams.In the Analyze phase, a prioritization matrix and a Pareto chart were used for root cause analysis followed by capability to check the performance of selected causes and to calculate their sigma level.The Improve phase included educational lectures for the Hepatology residents to raise their awareness regarding EBM and Biologic Variations, in addition to designing a new lab request.

RESULTS: After implementing the selected solutions for the ALT/AST, and Urea and Creatinine tests ordering processes, the stability, capability and the Sigma levels were tested again. 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. However, no significant difference was found in the frequency of ordering Urea and Creatinine between before and after the improvement ($P > 0.05$).

CONCLUSION: From the present work it was concluded that conjoint meetings between physicians and laboratorians, including educational sessions, are essential for any possible improvement initiatives.Simple process change (such as modifying the lab request form) could be an important source of improvement and a cost reduction tool in the pre pre-analytical lab phase.Six Sigma methodology is a powerful improvement / cost reduction tool that can be used in the healthcare sector in general and in the medical laboratories in particular.

B-206

A Shared Diagnostic Stewardship Approach to Autoimmune Encephalopathy Send-Out Testing

C. N. Sharp[1], P. Muluhngwi[2], A. Fletcher[1], M. Linder[1], J. Snyder[1], S. Jortani[1]. *[1]University of Louisville, Louisville, KY, [2]Northwestern University, Chicago, IL*

Background: As part of our laboratory testing utilization at University of Louisville Hospital (ULH), any send-out test ≥$500 must be assessed by the clinical chemistry fellow or pathology resident on-call for clinical necessity and as a way to provide consultation to the ordering physician on appropriate test utilization. In 2018, we noticed a prominent increase in the volume of panels for autoimmune encephalopathy (AE) and related disorders being ordered. This was met with considerable cost to the hospital, a lack of consensus on which panels should be ordered, and poor understanding of the clinical utility of these panels. These challenges presented an ideal project for our newly-formed diagnostic stewardship committee (DSC) at ULH. Through collaboration with the DSC, we were able to form a sub-committee with the Department of Neurology to develop an algorithm-based approach for ordering of these AE panels. Our goals were to improve diagnostic utility for the betterment of patient care, improve test utilization, and decrease cost associated with panel ordering.

Methods: Prior to involvement of the DSC, we analyzed the number and cost of all AE panels ordered in 2018. We also calculated the number of positive panels and reviewed patient data to determine if a positive panel corresponded with a clinical outcome. After presenting our data to the DSC, an evidence-based approach was taken in collaboration with the Department of Neurology to develop an algorithm for AE test ordering. Results of tests were analyzed for true positive rate, defined as a patient testing positive for an antibody with known etiology or association with AE based on result interpretation provided by Mayo Clinical Laboratories. The total cost of tests was also analyzed before implementation of the algorithm (January 1, 2019-July 31, 2019) and post-implementation (August 1, 2019-December 31, 2019).

Results: In 2018, 77 AE panels were ordered with an estimated charge to the laboratory of $137,510. The true positive rate was 10%. Based on these test ordering patterns, we projected that 120 tests would be ordered in 2019, totaling >$200,000. Prior to the implementation of the algorithm in 2019, 55 tests were ordered, totaling $105,210. Total true positive rate was 3.6%. After implementation of the algorithm, 23 tests were ordered, totaling $50,220. The true positive rate was 13%.

Conclusion: Using a shared diagnostic stewardship approach, we were able to successfully develop an evidence-based algorithm for guiding physicians for AE panel

ordering. Comparing test ordering patterns pre and post-implementation in 2019, our true positive rate more than tripled (3.6% to 13%), indicating that our algorithm was able to successfully identify the at-risk population for development of AE disorders. This was met with a >50% decrease in the number of tests ordered, and a total cost savings of $54,990 compared to the first half of 2019.

B-207

Utilization Review and Intervention to Reduce Unnecessary Vitamin B12 Tests in a Tertiary Care Academic Hospital

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Background: Vitamin B12 deficiency is frequently suspected upon discovery of macrocytic anemia or presentation of neurological impairment. Guidelines are unclear as to who should be tested and how frequent repeat testing should be done on B12 deficient patients. This study sought to identify the order patterns and apply a middleware rule to reduce unnecessary B12 tests in a tertiary care academic hospital. **Methods:** Data was extracted from the laboratory IS (Information System) for the period of 2016-2018 and analyzed for trends related to the patient, ordering physician, and hospital location. To limit the ability to perform a subsequent B12 test, rules were implemented in the cobas® IT middleware (Roche Diagnostics, Laval, Quebec, Canada) to restrict repeat testing within 90 days of a previous test result. **Results:** Annual B12 test utilization remained relatively the constant with approximately 6000 tests per year with a distribution of 54.2%, 38.0% and 7.8% from the Outpatient (OP), Inpatient (IP) and Emergency Room (ER) areas, respectively. In a different approach, retesting of the same patient was explored as a source of unnecessary B12 testing. In the 5 highest utilization areas representing the OP and ER, the average time of repeat testing was typically >6 months (range: 192-299 days). Interestingly, the average time in the 5 highest IP areas was often <3 months (range: 49-89 days). In the absence of a computerized protocol at the level of order entry either within the hospital or laboratory IS, a middleware algorithm was developed to automatically reject a B12 test if a normal result had been previously reported for a patient within 90 days. The algorithm was implemented in January 2020 and expected to reduce B12 testing by 8.6% for a modest savings of approximately CA$ 2000 per year based on the current Government of Ontario Schedule of Benefits. **Conclusion:** While B12 testing would be ordered in cases of suspected deficiency, repeat testing in <3 months appeared to be a significant source of unnecessary testing. Since physicians and healthcare providers may be too busy or find difficulty to search for the availability a previous result in the information system, a forced function rule within the laboratory is an effective intervention to reduce unnecessary repeat testing.

B-210

Large-Scale Retrospective Analyses of the Effect of Iron Deficiency Anemia on Hemoglobin A1c Levels

G. W. Pratt[1], C. Bi[2], M. H. Kroll[2], L. V. Rao[1]. *[1]Quest Diagnostics-North Region, Marlborough, MA, [2]Quest Diagnostics, Secaucus, NJ*

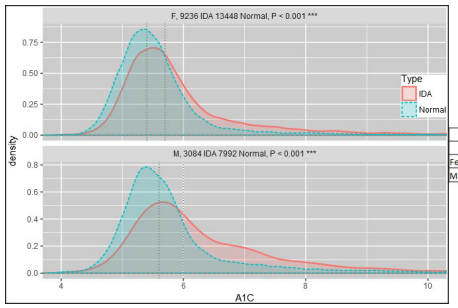
Introduction: Testing for hemoglobin A1c (HbA1c) is recommended for monitoring diabetes, but multiple conditions affect HbA1c levels. Any conditions that affect red cell turnover, such as iron-deficiency anemia (IDA), can lead to spurious HbA1c results. Reports on how IDA affects HbA1c levels are contradictory and use a small number of study participants with a spurious increase in values. To better understand the association between HbA1c levels and IDA, we conducted a retrospective study of HbA1c test results from a large reference laboratory.

Methods: Test results for HbA1c levels were retrieved from the Quest Diagnostics database for 2018 through 2019. Over 12,000 patients with IDA and 21,000 patients without IDA were analyzed to evaluate the effect of IDA on HbA1c measurements. Patients were classified as having IDA if samples with below, the age-based reference range for serum iron, ferritin, or transferrin iron saturation (FE sat), and above age-based reference ranges for transferrin iron-binding capacity (TIBC), or transferrin (TF) levels. Kruskal-Wallis statistical analysis method was used to test whether the two samples follow the same distribution and significance.

Results: Data was separated by sex as many of the tests have gender-specific reference ranges. For female patients, the median HbA1c level was 5.7% among IDA classified patients and 5.4% among normal samples (P<0.001). For male patients, the median HbA1c level was 6.0% among IDA classified patients and 5.6% among normal samples (P<0.001).

Conclusion: These results indicate that patients classified as IDA can have increased HbA1c levels compared to patients without IDA. When using HbA1c levels to monitor diabetes, healthcare professionals should consider IDA status before making any therapeutic decisions.

Figure 1. Population density distribution and median HbA1C levels in patients with IDA (red) and normal (blue). Top (Female) and Bottom (Male).



HbA1c Median (# of data points)			
	Normal	IDA	p value
Female	5.4 (13448)	5.7 (9236)	<0.001
Male	5.6 (7992)	6.0 (3084)	<0.001

B-211

Association of High AST and ALT Values with Hemoglobin A1c Levels

G. W. Pratt[1], C. Bi[2], M. H. Kroll[2], L. V. Rao[1]. *[1]Quest Diagnostics-North Region, Marlborough, MA, [2]Quest Diagnostics, Secaucus, NJ*

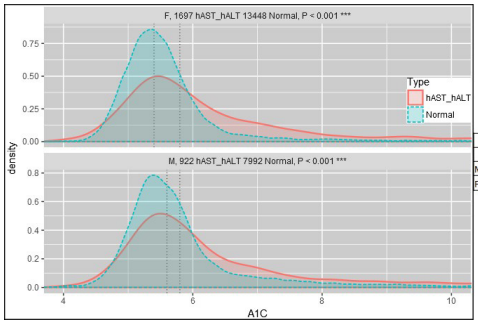
Introduction: HbA1c is the gold standard for the measurement of glycemic control in patients with diabetes mellitus type2. There is disagreement in the literature on the relationship between HbA1c and liver disease. Case reports have shown lower HbA1c levels and liver disease; other studies have shown higher HbA1c levels and high levels of markers that indicate liver problems (AST/ALT). To explore the association of liver dysfunction on glycemic control, we retrospectively examined HbA1c levels in patients with and without high AST/ALT levels from a large reference laboratory.

Methods: HbA1c **t**est results were analyzed from the Quest Diagnostics database from 2018- 2019. Over 2,500 patient samples with both high AST/ALT levels (above age-specific reference ranges) and over 21,000 patient samples without high (do not exceed the upper limit of age-specific reference ranges) AST/ALT levels, iron deficiency anemia, or chronic kidney disease were analyzed. Kruskal-Wallis statistical analyses method was used to test whether the two samples follow the same distribution and significance.

Results: Patients with high levels of AST/ALT exhibited higher HbA1c when compared to patients with normal levels. For female patients, the median HbA1c was 5.8% among high AST/ALT samples and 5.4% among normal samples (P<0.001). For male patients, the median HbA1c level was 5.8% among high AST/ALT samples and 5.6% among normal samples (P<0.001).

Conclusion: Higher AST/ALT enzyme levels are associated with higher HbA1c measurements. Thus, when using HbA1c levels to monitor glycemic controls and diabetes, liver enzyme status should be considered before making any therapeutic decisions.

Figure 1. Population density distribution and median HbA1C levels (vertical dotted lines) in patients with high AST/ALT (red) and normal (blue).



HbA1c Median (# of data points)		
	Normal	hAST+hALT
Male	5.6 (7992)	5.8 (922)
Female	5.4 (13448)	5.8 (1697)

B-212

Investigation of Distributions of Results for Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LD) Cancelled due to Hemolysis, and Recommendations for Change of Cancellation Policies at Our Institution

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BACKGROUND: Our hospital laboratory uses Roche Cobas technology for chemistry tests. Our policy for cancellation of AST and LD results due to hemolysis as a positive interferent has been very conservative, using hemolysis index cutoffs documented to produce >10% interference according to test kit inserts (for AST, HI>40; for LD, HI>15). These HI cutoffs foster high test cancellation rates: in one recent month, for AST, the cancellation rate was 4.9% of submissions; for LD, the cancellation rate was 8.3%. For many end users, the context of measurement of these analytes is only to determine whether the concentration is elevated (e.g., for infusion center patients, for clinical trials patients). In this context, our cancellation policy may often be withholding useful information for samples that, despite hemolysis, produce results within the reference interval. We therefore considered a change in policies to enable reporting of such results. The fraction of cancelled results that would be affected, however, was unknown. Our objectives were to document the distributions of results cancelled due to hemolysis for AST and LD, and, on that basis, potentially to recommend changes to our cancellation policies. **METHODS**. For tests cancelled due to hemolysis, our hospital information system (EPIC) retains information on HI, but does not retain the information on the measured value of the analyte that was cancelled. This information had to be retrieved manually from Roche instruments using the instruments' Data Manager. This required line by line examination of AST or LD test results to identify samples flagged for hemolysis, followed by examination of individual instrument sample reports to obtain the measured AST or LD. Analysis of distributions of cancelled AST and LD results was performed using Excel software. For comparison, distributions of reported AST and LD results were obtained directly from EPIC using pre-existing EPIC report templates. **RESULTS**: Data for reported results were obtained for recent one-month intervals (Nov or Dec, 2019). Among reported results, 73.8% of the total number of AST results (n=6651) were within the AST reference interval (7-42 U/L), and 35.7% of the total number of LD results (n=990) were within the LD reference interval (125-240 U/L). Among cancelled results for AST and LD obtained manually as described above, 60.6% of the cancelled AST results (n=207) were within the AST reference interval, and 11.8% of the cancelled LD results (n=203) were within the LD reference interval. **CONCLUSIONS**: Reporting of AST and LD results with hemolysis but lying within their reference intervals would reduce the total number of call-backs and recollections by approximately 60% and 12% for AST and LD, respectively. For many end users, such reports of an "at most" concentration for these analytes may be sufficient information despite positive interference. We therefore proposed to our laboratory administration that AST and LD results for hemolyzed specimens within the reference interval should be reported as follows: "*Hemolyzed specimen. Result shown is within reference interval. However, it is likely to include a component of positive interference due to hemolysis. Recommend redraw if clinically indicated*."

B-216

RF Interference in Lateral Flow Assays

M. Halat, E. Gravens. *Omega Biologicals, Inc., Bozeman, MT*

Background: A lateral flow immunoassay is often used as a quick and affordable means to aid diagnosis and decision making in patient care. Accurate results are crucial as a positive result may prompt further testing. Heterophilic antibodies may be present in patient specimens and can cause interference through non-specific binding in immunoassays. Rheumatoid Factor (RF) is one example; it can be present in the majority of Rheumatoid Arthritis (RA) patients. This study examines the performance of a lateral flow assay in the presence of RF. The assay was chosen for its clinical relevance; it is designed to identify elevated cardiac markers in Acute Myocardial Infarction (AMI) patients. **Methods:** Acquired an FDA registered, CE marked lateral flow immunoassay for determination of Cardiac Troponin I (cTnI), Creatine Kinase MB (CK-MB), and myoglobin with different cutoff concentrations. RF positive serum specimens (n=26) were obtained, nine male, seventeen female, ages 32-99, with RF titers ranging from 80-9375 IU/mL. Eight plasma specimens from donors with a RA diagnosis were obtained, all female, ages 46-70, with RF titers ranging from 107->600 IU/mL. Patient specimens were tested according to manufacturer's recommendations; 80 µL of serum or plasma were added to the sample well; results were read after 15 minutes. Specimens suspected of false positive results were retested with HeteroBlock®, a commercially available blocking reagent. HeteroBlock was prepared at 2 mg/mL concentration, 10 µL of the HeteroBlock solution were added to 80 µL of serum or plasma, mixed well and allowed to stand for five minutes at room temperature before testing. Positive and negative control samples were acquired to verify kit performance. Control samples were prepared with and without HeteroBlock and run side-by-side. **Results:** Of the 26 RF positive serum specimens, seven specimens tested positive for one or more cardiac biomarkers. One specimen tested positive for all three biomarkers; when retested with HeteroBlock the signal was reduced in intensity. Four specimens tested positive for CK-MB; when retested with HeteroBlock the signals were eliminated. Two specimens tested positive for myoglobin; when retested with HeteroBlock one signal was reduced in intensity, one signal was unchanged. Three of the eight RA patient plasma specimens tested positive for cTnI and CK-MB. The three positive plasma specimens were retested with HeteroBlock. The addition of HeteroBlock eliminated the positives in all three plasma specimens. A negative and positive control were tested with and without HeteroBlock. The addition of HeteroBlock did not change the results of the positive or negative controls supporting the expectation that HeteroBlock does not affect true positive results. **Conclusion:** Lateral flow immunoassays may be susceptible to interference by RF leading to false positive results. The assay surveyed used three unique biomarkers with different cutoff levels; all three biomarkers revealed some risk for interference. Specimens that caused interference for one biomarker did not necessarily interfere with other biomarkers. This study reinforces the need for vigilance regarding the potential for false positive results caused by heterophilic antibody interference.

B-217

Successful Conversion to a High-Sensitivity Troponin I Assay

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Background: Our lab recently transitioned from the well-accepted sensitive cardiac troponin I (cTnI) assay on the Siemens 1500 Dimension Vista platform to the high-sensitivity cTnI (hs-cTnI) troponin I assay on the Atellica IM Analyzer (Atellica IM TnIH). This assay reportedly meets specifications for a hs-cTn assay (>50% detection in a healthy reference population with sex-specific 99th percentiles and ≤10%CV). The announced move to the hs-cTn assay raised clinician concerns about increased reactive rates and sex-specific versus overall cutoff points. To support successful transition, the lab collaborated with clinicians to verify hs-cTnI methods with the previous assay and educate.

Methods: A method validation procedure between the previous assay (VISTA cTnI) and the new hs-cTn assays (Atellica IM TnIH and Vista TNIH) was developed. Measures included repeatability, precision (including low-end), internal quality control (IQC) validation (BioRad), quantification limits, and carry-over/contamination assessment. An inter-sample method comparison was conducted between the old and new assays over the entire measuring range. Additional analyses included comparisons targeted to values near the 99th percentiles (positivity thresholds), analysis in an older patient population (>75 years), and interpretation of results according the sex-specific thresholds versus overall. In parallel, education for physicians was launched a year ahead of the transition, including identification of clinical advocates, information on reporting whole numbers (ng/L) and gender-specific values, presentations, and an email campaign for primary requesters of cTn testing (ED physicians, hospitalists, and cardiologists) before the general dissemination. All questions were responded to. The remaining staff was notified of the move two weeks in advance. A review to assess usage and impact was conducted one-month post-implementation**.**

Results: Repeatability, internal quality control, and precision profile results were in line with the manufacturers stated performance for hs-cTnI assays, including CV's of IQC for values close to the decision threshold and the quantification limit. Reproducibility. Vista TNIH (n=31): 47.6 ng/L CV2.3%; 4137 ng/L CV1.9%; 22381 ng/L CV3.2%. Atellica IM TnIH (n=30): 49.75 ng/L CV1.83%; 16784.73 ng/L CV0.93%. Within lab (n=34) Vista TNIH: 42.5 ng/L CV4.7%; 3049 ng/L CV 4.0%; 19705 ng/L CV5.3%. Atellica TnIH: 36.17 ng/L CV4.89%; 4358.42 ng/L CV3.34%; 11583 ng/L CV3.23%. Excellent agreement between hs-cTnI assay values were observed between the two mirrored Atellica modules including samples from the older cohort (>75y) and values around the decision threshold. Good clinical agreement between hs-cTnI assays and the previous VISTA cTnI was seen. Vista TNIH=1.0331 x Vista cTnI-23.278, R^2=0.9852; Atellica IM TnIH =1.0106 x Vista cTnI-31.175, R^2=0.9932; Atellica IM 2 TnIH=0.9658 x Atellica IM 1 TnIH+3.0998, R^2=0.9997. The ordering pattern analyzed post-implementation revealed reductions in number of cTn orders in

patients >75y; a stable pattern for male positivity rates; an increase in positive results in women, including those >75y (expected for the lower, female-specific threshold). These results allayed physician concerns over any increases in positive tests (especially in older patients), or greater need for cardiology consultation.

Conclusion: Evaluation and validation of the performance of hs-cTnI assays, along with physician education in advance of the transition, can help the laboratory achieve successful implementation; clinical staff readily adopted whole numbers and sex-specific thresholds.

B-218

A Prototype Study to Support Development of the New Cobas® Pure Integrated Solutions

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Background:

cobas® pure integrated solutions is a new generation clinical chemistry and immunochemistry analyzer in development by Roche Diagnostics. **cobas pure** is designed for the low to mid volume laboratory settings as a main analyzer in small labs, dedicated analyzer for specific panels, STAT analyzer or back up analyzer to mid to high volume analyzer**s**. **cobas pure** will be available as stand-alone clinical chemistry analyzer including electrolytes (ISE) on a 1.2 m^2 footprint, as stand-alone immunochemistry analyzer on a 1.2 m^2 footprint and as an integrated solution on a 2 m^2 footprint. **cobas pure** will have a throughput of up to 450 photometric, 450 ISE and 120 immunochemistry tests per hour. While still being in the prototype phase of development, **cobas pure** was placed in one of the nine interconnected labs of the Central Institute of the Hospitals, located in the hospital of Visp in Wallis, Switzerland. Goal was to test the system under routine-like conditions to gain insights of the overall performance and ease of use of the analyzer in the hands of intended-users.

Methods:

Analytical system performance was tested using the CLSI EP05-A3 precision protocol over 21 days.The overall analyzer functionality was tested using routine simulation experiments designed to test the interaction of hardware, software, chemistry, biological samples and users. Those protocols were designed to stress test the system under routine-like conditions for a prolonged.A total of 23 applications for ISE, clinical chemistry and immunochemistry assays were used: Cl, K and Na serum and urine applications on the ISE unit; ALT, AST, Chol, Ca, Phos, CRP serum applications, Crea, Gluc serum and urine applications on the clinical chemistry analytical unit (c 303) and Pro-BNP, FT4, TSH, Ferr, Fol and HIV on the immunochemistry analytical unit (e 402).

Results:

From the 21-day precision measurements using quality controls as sample material, the within-lab precision CVs of the 6 ISE applications were found to be below 2%, the clinical chemistry photometric assay CVs were below 2.5% and the immunochemistry CVs were below 6%.During the routine simulation precision experiments, 198 CVs were generated for up to 23 applications. The median CV of 0.8% reflects the very good system performance observed under routine-like conditions over these experiments with up to 5h duration.For the method comparison download experiments, routine left-over samples were re-measured on cobas pure integrated solutions and the results compared with the original cobas e 411 and cobas Integra 400 plus routine results. From the resulting 19 method comparison regression analyses, slopes ranged from 0.82 to 1.13.

Summary:

Overall, the study showed that the **cobas pure** prototype already demonstrated good reliability and very good analytical performance under routine-like conditions. In addition, the use of the c pack and e pack green reagent carriers that have longer on board stability and less calibration frequency in an integrated analyzer for small to medium size laboratories was very promising for the future integration of the **cobas pure** integrated solution into existing laboratory infrastructure.

B-219

Data for Laboratory Workforce Development: What Do We Have? What Do We Need?

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Background: Clinical and public health laboratories face an array of challenges in workforce development, including recruitment, retention, and staff training and professional development. While there is an abundance of awareness and extensive, first-hand experience with these challenges within the laboratory community, it is unclear exactly what, and how much, hard data exist regarding the things we think we "know" about laboratory workforce development. **Methods:** To answer this question, we conducted an environmental scan to uncover existing data and gather new data to better understand the laboratory workforce and workforce development challenges. The scan consisted of a literature review as well as interviews, focus groups, and other discussions with CDC staff, external partners, and members of the clinical and public health laboratory communities. **Results:** The literature review assessed the amount, quality, and scope of available information related to the workforce and challenges associated with the education, recruitment, professional development, and retention of laboratory professionals. Out of 4,000+ data sources uncovered from searching key words and phrases within four scientific databases [Medline (OVID), Embase (OVID), CINAHL (Ebsco), and Scopus] and the internet, 243 sources were eligible for inclusion in the literature review by meeting criteria related to topic area, audience, and publication year. The largest proportion of published data— and the most robust data — for both clinical and public health laboratory workforces related to the categorization of each workforce at the national level, including data on demographics, educational attainment, and type of employer. However, data were scarce for most other topics, including the awareness and perceptions of careers in laboratory science, best practices in retention, and the root causes of the workforce shortage. We also present themes from interviews, focus groups, and other discussions conducted in 2018-2020 with members of the laboratory community and other stakeholders regarding workforce development needs and challenges, particularly in the training and professional development of current laboratory professionals. These themes include: preferences for learning modalities; facilitators and barriers to accessing available training; and current or expected needs for training, especially regarding emerging trends in laboratory science, cross-cutting topics such as laboratory quality and safety, and 'soft skills' such as leadership. **Conclusion:** In total, the environmental scan demonstrates several areas for improvement in the gathering and measurement of workforce development data. This scarcity of information prevents the development of data-driven strategies and efforts that could be used to better identify and address critical needs in the creation and maintenance of a well-trained and skilled laboratory workforce. The findings from the environmental scan will help enhance CDC's engagement with partners in developing goals, programs, and resources to address laboratory workforce development needs at the national level.

B-220

Evaluation of An Automated Tool to Calculate Measurement Uncertainty According to New ISO/TS 20914 "Practical Guidance for the Estimation of Measurement Uncertainty"

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Objective:

Bio-Rad's Unity Real Time 2.0 (URT2) Quality Control Data management software recently added functionality providing a Measurement Uncertainty (MU) report and 3 different ways to calculate MU. How well do these reports compare to the new ISO/TS20914 "Practical guidance for the estimation of measurement uncertainty"?

Background:

ISO15189 requires MU to be calculated for all analytes (Section 5.5.1.4). "The laboratory shall also define the performance requirements for MU and regularly review the MU estimates." Until 2019 there was no ISO guidance on how to calculate MU, which resulted in numerous formulas used worldwide. While the ISO20914 formulas are straightforward, they are manually performed which can lead to data transposition, inconsistent selection of data for calculation, and other errors. An automated process was needed to consistently extract data, accurately perform the calculations, and present the results in a convenient report.

Methodology:

The formulas in the ISO/TS20914 guidelines are compared to the URT2 Measurement Uncertainty report formulas. Additionally 3 practical worked examples from the ISO/TS20914 document (Table A1, A2 and C2 from the Annex A and C) are entered into the URT2 software and results are compared.

Validation:

Example	Formula	Calculated example
ISO/TS20914 Table A2 Expanded without U_{cal} provided	$U(y) = 2 \times \sqrt{(u^2_{Rw})}$	Level1 Level 2 Level 3 4.2% 3.7% 5.2%
URT2 Expanded without U_{cal} provided	U = 2 x SD	Level 1 Level 2 Level 3 4.19% 3.74% 5.16%
ISO/TS20914 Table A1 Expanded with U_{cal} provided	$U(y) = 2x \sqrt{(u^2_{Cal} + u^2_{Rw})}$	Level 1 Level 2 Urine IQC 1.6% 1.5% 2.8%
URT2 Expanded with U_{cal} provided	$U = 2 \times \sqrt{(SD^2 + Cal\ U^2)}$	Level 1 Level 2 Level 3 1.64% 1.50% 2.82%
ISO/TS20914 Table C1 Expanded with bias correction	$U(y) = 2 \times \sqrt{(u^2_{bias} + u^2_{cal} + u^2_{Rw})}$	Level 1 1.6%
URT2 Combined expanded Uncertainty (+Interlaboratory bias)	$U = 2 \times \sqrt{(SD^2 + (Bias/\sqrt{3})^2 + SDBias^2)}$ (Ref COFRAC SH GTA 14 guideline)	Level 1 1.99%

The formulas to calculate the MU with and without the uncertainty of the calibrator (U_{Cal}) are the same in URT2 and ISO/TS20914 and provide the same results.

Calculating MU when correcting a bias in the ISO/TC20914 does not correspond to the URT2 calculation of MU with Interlaboratory bias, which is currently based upon the French COFRAC SHGTA14 guideline. This specific formula will be used infrequently as bias correction is not routine practice in the laboratory.

Conclusion:

URT2 provides a useful tool to automatically calculate MU from QC data captured as part of normal lab routine. The recommended formula for expanded MU provides identical results as the worked examples in the ISO standard.

B-221

Storage Conditions of Fecal Swabs for the Diagnosis of Vancomycin-Resistant Enterococci (VRE)

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Background: Enterococci are part of normal gut flora but have emerged as important nosocomial pathogens due to their resistance to antimicrobial agents. Vancomycin-resistant enterococci (VRE) infection is associated with a higher mortality rate. Real-Time PCR (qPCR) has become a robust technique to improve diagnosis and storage conditions of the sample can influence the test result. For this reason, it is necessary to know the storage conditions of fecal swabs to avoid false results in the diagnosis of VRE.

Methods: This study was conducted with 47 fecal swabs (ESwab®, Copan, Italy) that were previously analyzed and resulted negative for VRE. All the samples were pooled, mixed and divided into four different aliquots. Three of them were spiked, individually, with cultures of CECT 5253 *Enterococcus faecium vanA*, NCTC 12201 *Enterococcus faecalis vanA* and CECT 8120 *Enterococcus faecalis vanB*. The concentration reached was 10 CFU/rxn, 7.5 CFU/rxn and 30 CFU/rxn, respectively. The last aliquot, not spiked, served as a negative control. All qPCR assays were performed with the VIASURE *Vancomycin resistance* Real Time PCR Detection Kit for use with the BD MAX™ System (Certest Biotec, Spain) and run on the BD MAX™ System. The tested storage conditions of the four samples (three positives and one negative) before processing were: room temperature (22°C -25°C) at 0 hours, 24 hours and 48 hours; in a refrigerator (2ºC -8ºC) at 144 hours (6 days); and frozen (-20ºC) at 196 hours (8 days). Each sample was tested six times in duplicate by condition.

Results: All positives samples were detected in all conditions, except at room temperature at 48 hours. The best mean Cq value was obtained for room temperature at 0 hours, but there was no more than 1.5 Cq of difference between all conditions. There was no amplification in the negative control in any condition.

Conclusions: Fecal swabs for VRE diagnosis can be stored at 22°C -25°C for up to 24 hours, 2 to 8°C for up to 144 hours (6 days) or frozen at -20°C for up to 196 hours (8 days).

B-222

Evaluation of Different Storage Conditions of Nasopharyngeal Swabs for the Detection of Bordetella spp

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Background: *Bordetella* species can cause respiratory disease from nonspecific symptoms to whooping cough or even death, such as that caused by *B. pertussis*. It is important to differentiate *B. pertussis* from other *Bordetella* spp, including *B. holmesii* and *B. parapertussis*, not only to assess the vaccine's efficacy but also to determine the most appropriate antibiotic treatment. Real-Time PCR (qPCR) of nasopharyngeal specimens is often used for the diagnosis of *Bordetella* spp. Because storage conditions for nasopharyngeal swabs (NPS) may affect the diagnosis of *Bordetella*, a study of sample stability is needed.

Methods: Twenty-two nasopharyngeal swabs (NPS) in viral transport medium (Vircell, Spain) were used for this study. After verifying that all the NPS were negative for *Bordetella*, they were mixed in one pool and divided into four aliquots. One of them was not spiked and served as a negative control. The other three aliquots were spiked with NCTC 12912 *B. holmesii* (1 CFU/rxn), NCTC 5952 *B. parapertusis* (2 CFU/rxn) and NCTC 10379 *B. pertussis* (1 CFU/rxn), respectively. All qPCR assays were performed with the VIASURE *Bordetella* Real Time PCR Detection Kit for use with the BD MAX™ System (Certest Biotec, Spain) and run on the BD MAX™ System. Four different storage conditions (before processing) were evaluated for each sample: room temperature -RT- (22°C -25°C) at 0 hours, RT (22°C -25°C) at 24 hours; in a refrigerator (2ºC -8ºC) at 144 hours (6 days); and frozen (-20ºC) at 196 hours (8 days). Twelve replicates were tested for each sample/condition.

Results: There were no significant differences among the conditions RT 0 hours, RT 24 hours and in a refrigerator 6 days in terms of mean Cq values. The frozen condition, 8 days, obtained the best mean Cq value, improving with respect to the RT condition 0 hours. There was no more than 1 Cq of difference between all conditions. No amplifications were detected in the negative control.

Conclusion: the NPS in viral transport medium for the detection of *Bordetella* spp can be stored at 22°C -25°C for up to 24 hours, 2 to 8°C for up to 144 hours (6 days) or frozen at -20°C for up to 196 hours (8 days).

B-223

Guideline Compliance in Multiple Myeloma Testing: An Audit of Orders by Specialty

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Background: Monoclonal gammopathy of unknown significance (MGUS), smoldering multiple myeloma (SMM), and multiple myeloma (MM) are progressive plasma cell disorders (PCD). The early symptoms may be non-specific, and delayed diagnosis can lead to chronic complications and increased downstream costs. Combinations of laboratory tests including serum or urine protein electrophoresis (sPEP/uPEP), immunofixation (sIFE/uIFE), and free light chains (sFLC/uFLC) are requested to diagnose and monitor PCD. The International Myeloma Working Group guidelines recommend sPEP, sIFE and sFLC for diagnosis of PCD (other than AL amyloidosis). Only 6% of MM diagnosis testing is guideline compliant (GC). Various combinations of tests are recommended to monitor PCD or evaluate depth of treatment response.

Methods: We conducted an audit of ordering patterns within St. Elizabeth Healthcare (SEH) by querying Epic Beaker for tests requested November 2018 to November 2019. SEH is an integrated healthcare delivery system, operating 115 primary care and specialty locations in Kentucky, Indiana, and Ohio. Data were segmented by ordering physician specialty. Guideline compliance was calculated by specialty, assuming that non-oncology clinicians were testing to diagnose MM, while oncology-initiated testing was predominantly for monitoring purposes.

Results: A total of 11 unique testing combinations were ordered during 3,945 patient encounters. Non-oncology and oncology providers ordered testing during 2,189 and 1,756 patient encounters, respectively. Nephrology and neurology requested orders were 26% and 17% GC, respectively. Primary care practitioners demonstrated only 12% GC.

Conclusion: The findings highlight a gap in test order optimization among healthcare practitioners. The laboratory can improve GC test ordering by leveraging information system tools to better understand test utilization patterns and sharing the data. The oversight of multi-specialty lab stewardship committees is invaluable when considering solutions. Approving GC test panels, modifying order preference lists, and providing decision support tools are strategic opportunities for stewardship bodies to improve diagnostic management.

Volumes by Clinical Specialty						
Test(s)	Total Volume (all specialties)	Heme-Onc	Primary Care	Nephrology	Neurology	Other
sPEP:	503	17	139	98	106	143
sPEP + sFLC:	185	147	8	7	2	21
sPEP + sIFE:	1320	425	379	174	192	150
sPEP + sIFE + sFLC:	1354	1003	76	197	63	15
sPEP + uIFE + uFLC:	18	0	6	10	0	2
sFLC:	161	82	11	57	5	6
sPEP + sIFE + sFLC + uIFE + uFLC:	95	11	4	66	2	12
sPEP + sFLC + uIFE + uFLC:	7	0	0	4	0	3
sPEP + sIFE + uIFE + uFLC:	94	0	4	63	1	26
sFLC + uIFE + uFLC:	5	0	1	4	0	0
uIFE + uFLC:	203	71	17	88	1	26
Totals:	3945	1756	645	768	372	404

B-224

Dramatic Decreases in Unnecessary Repeat QC and Calibrations through Application of Six Sigma to High Volume Lab Tests

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Background and aim:

Six sigma is a highly utilized, data-driven approach used by organizations internationally and represents a level of reproducibility and quality for manufacturing and transactional processes that have attained an error or "defect" rate of ≤ 3.4 per million opportunities. This is clearly a low rate of error, but when dealing with the health and wellbeing of patients error can never be too low. The Cedars-Sinai Core laboratory recently took up a project to obtain Six Sigma laboratory certification. This entails training of lab leaders in Six Sigma principles, evaluating tests in the lab for their sigma metrics, and obtaining at least 20 tests performing at six sigma or above. Ultimately, we wanted to determine if modified QC rules based on individualized test sigma metrics would significantly reduce unnecessary repeat QC and calibrations by laboratory staff.

Methods:

Sigma metrics are calculated as follows: (TEa - Bias)/ CV, where TEa = total allowable error, Bias = bias of our QC results as compared to peer means, and CV = coefficient of variation. In all, >50 chemistry and immunoassay tests were evaluated for their sigma performance. Westgard and Westgard have described which QC rules (number and type) are necessary for test monitoring based on the sigma metrics, and observed inaccuracy and imprecision of a test. These recommendations can be visualized with "Westgard Sigma Rules" or OPSpecs charts. Previously Cedars-Sinai implemented the same set of Westgard rules for all routine quantitative tests on every redundant instrument (1:2s, 1:3s, 2:2S, R:4S, 4:1s, 10x•••). At the beginning of January 2020, we selected 18 out of 19 routine chemistry tests (focusing on one chemistry instrument) performing at > 4 sigma (15 of which were > 6 sigma) and reduced the number of QC rules as follows: 4 sigma: 1:3s, 2:2s, R:4s, 4:1s; 5 sigma: 1:3s, 2:2s, R:4s, 6 sigma: 1:3s, within our QC monitoring system (BioRad Unity). We then compared the repeat QCs and calibrations for January 2020 vs. December 2019.

Results:

In December 2019, 35 repeat calibrations, and 162 repeated QCs of the 18 selected high performing tests were observed. In January 2020 after reducing our QC rules, as previously described based on sigma performance, only 7 recalibrations and 62 repeated QCs were observed. Recalibrations dropped by 80% and repeated QC dropped by 62%.

Conclusion:

Once Westgard QC rules were updated for 18 tests performing at ≥ 4 sigma drops in recalibrations (80% down) and repeat QC (62% down) were observed over a 30-day period post change. This indicates that cost and labor savings are incurred with tailoring Westgard rules to sigma levels (which we will calculate). The more high-performing tests run in a clinical lab the better, since low-performing tests (e.g., ≤ 3 sigma) require even more QC rules and tighter control. Further, it is anticipated that with time additional reductions in repeat QC and calibration rates may be observed as lab staff grow more comfortable with these updates. We plan to reassess QC and calibration rates post-change at 6 months (and across multiple instruments).

B-225

Economic Impact of Using a Heel Incision Device for Sample Collection by Heelstick in Three Newborn Screening Public Programs in Mexico

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Background: Newborn Screening (NS) is a pediatric practice to prevent disability at birth and is a right of every newborn in Mexico. It's performed by taking a capillary blood sample from the newborn by heel stick with a lancet or a heel incision device (HID). In most of the NS public programs in Mexico, conventional lancets or puncture devices (PD) are still used, although they require a greater number of punctures and the procedure is more traumatic than with HID. There is evidence that when using HID instead of PD, pain, puncture frequency, duration of procedures and tissue damage are significantly reduced. **Objective:** To carry out a meta-analysis to determine the health and economic impact when using HID instead of PD for heel capillary sampling in 3 public NS programs. **Methods:** A meta-analysis of data published in the literature on the efficacy for heel sampling performed by different nurses with HID and PD, was performed using a random effect model. A complete economic assessment regarding Cost-Effectiveness, was carried out using a decision model. The outcome was defined as the repetition rate for sampling by heel stick in newborns that require blood sampling, either for NS or for any other clinical study. Direct and associated costs were considered. An univariate sensitivity analysis on HID price and on effectiveness measurements was performed. Mathematical models were applied to determine the Incremental Effective Cost Ratio (ICER), the chosen option would be the device that showed the lowest ICER among the assessed alternatives.The budget impact was calculated for up to 5 years with a cohort value of 10,000 punctures and the economic impact per year on three NS public programs (IMSS, ISSSTE and Secretaria de Salud), according to the number of births reported in 2016. **Results:** Data on 520 infants were evaluated, 259 sampled with PD and 261 with HID. 96.2% of HID procedures will require only one incision to obtain an adequate blood sample, compared to 68.0% of PD procedures. Although PDs are cheaper than HIDs, this meta-analysis shows that the total cost of puncture with HID is US $6.43 compared to US$8.43 with PD, given the greater effectiveness of HID. The lowest ICER obtained was US-$7.44 for HID. The annual budget impact in the three institutions indicates a saving of US$1,072,007 per year. **Conclusion:** Using HID in NS public programs in Mexico represents a cost-saving strategy, compared to PD. Savings could allow to expand or improve NS public programs. Beyond the economic results, the most important thing is to make the decision makers aware that each NS re-sampling could lead to the irreparable loss of the diagnostic opportunity.

B-227

The Usefulness of the Suppression Effect of Blood Glucose Rise Caused by Increasing the Production of Ketone Bodies by Taking MCT Oil

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Background: Medium-chain triglycerides (MCT) are composed of a glycerol backbone and three fatty acids that have an aliphatic tail of 8-10 carbon atoms. MCT oil is easily digested and absorbed by the body. Moreover, it efficiently restricts carbo-

hydrates by producing ketone bodies and does not easily accumulate in the body. In previous studies using a continuous glucose meter, it has been confirmed that MCT oil had an inhibitory effect of about 5%~20% on postprandial rise in blood glucose levels.

Aim: This study aimed to investigate the pathological condition responsible for the suppression of elevated blood glucose levels from changes in laboratory evidence due to MCT oil ingestion.

Methods:18 adult volunteers (2 men and 16 women) in their 10s-60s were orally loaded with 75 g glucose, and blood was collected at 0, 30, 60, 90, and 120 minutes, Oral Glucose Tolerance Test (OGTT).

The levels of glucose, ketone bodies, insulin, and 21 laboratory examination items were measured in a group in which the subjects took 15 mL of MCT oil before glucose load, and compared to 0-mL MCT oil group in which the subjects did not take MCT oil.

Results: The median relative ratio due to oil ingestion of change in glucose concentration was 15.4% (IQR: -4.3~28.0) at 60 minutes and 23.6% (1.9~31.9) at 90 minutes. In addition, the median rate of change in total ketone bodies decreased significantly in the 0 mL oil ingestion group to -24.4%(-40.7~11.1) at 30 minutes, -65.0%(-74.4~-57.1) at 60 minutes, -72.1%(-85.6~-55.7) at 90 minutes, and -74% (-84.6~-64.1) at 120 minutes, and the median rate of change in total ketone bodies in 15mL oil ingestion group was -14.7% (-31.7~17.0) at 30 minutes, -39.8% (-73.1~-7.1) at 60 minutes, -53.4% (-76.9~-22.4) at 90 minutes and -58.4% (-81.4~-19.1) at 120 minutes. In the group in which 15 mL of oil was taken, ketone bodies decreased after glucose load, but it was found that a certain concentration of ketone bodies remained. On the other hand, the insulin level was 10 to 13 times higher than normal after 30 minutes of glucose load, and decreased in accordance with the decrease in glucose concentration. However, there wasn't significant difference in the amount of insulin secreted depending on the presence or absence of MCT oil.

Discussion: Ingestion of MCT oil maintained total ketone bodies at a constant concentration, indicating suppression of blood glucose rise. However, the fluctuation of insulin level due to glucose load remained the same regardless of MCT oil ingestion. It is said that the insulin-like action of MCT oil is considered. It is thought that the long-term presence of ketone bodies in the blood due to β-oxidation of MCT oil continues activation of glucose metabolism, thereby suppressing an increase in blood glucose.

Conclusion: Ingestion of MCT oil can maintain glucose metabolism because total ketone bodies are in the blood for a long time, which is effective in suppressing postprandial rise in blood glucose.

B-230

Advanced Instrument-Guided Troubleshooting for Future Automated Analyzers

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Introduction:

Troubleshooting and maintaining automated immunoassay systems can significantly impact system downtime and the throughput of a lab. To perform troubleshooting and maintenance procedures, users are often required to reference printed maintenance procedures or troubleshooting guides, utilize tribal knowledge, or perform complex manual tasks. A consistent and simplified Help Wizard solution has been created to allow users to complete these tasks more efficiently on future automated immunoassay systems.

Method:

A *Wizard* is a workflow that guides users through the performance of procedures in a highly structured and consistent way. Wizards are especially useful for highly regimented and complex activities such as troubleshooting, maintenance, or for rare tasks requiring human intervention. A Wizard has been created for use on future analyzers where workflows are segmented into steps that are performed jointly by the system and the user.

Results:

Instrument features, such as hardware sensors, are used to identify analyzer status. For example, during a barcode read failure, the barcode scanner can be polled to determine whether the failure resides with the consumable, or the analyzer. Then, the hardware and software interact to either automatically complete a workflow step, or present relevant Help instructions needed by the operator to manually complete an action. Software is structured to provide a consistent user interface experience for the performance of all Wizards. Workflows are interactive and allow for branching depending on system status or user input.

Examples of Wizards and how they work:

1) Maintenance:

Running a cleaning panel is a weekly task that requires the user to input rack and tube information. The Wizard user interface includes fields for all information that the user must identify before loading the panel. Once the user inputs the required information, the system automates the remaining steps. Once complete, the instrument automatically documents performance of the task, date, time and operator in an electronic maintenance log.

2) Troubleshooting:

Failure to read a consumable barcode - Cameras prepare an image of the barcode for some consumables used by the instrument. When a camera fails to read a barcode, the Wizard allows the user to compare the barcode image with examples of good and bad barcode images. Troubleshooting is branched and directs the user to identify why the barcode read failure occurred. The user answers questions to be taken down the correct branch and resolve the issue. Once the end of the branch is reached, the issue is resolved and the user can continue running the analyzer.

Conclusion:

While it is preferable to fully automate all maintenance, error handling, and troubleshooting processes, tasks remain that require human interaction, input and judgement. The new advanced instrument-guided troubleshooting workflows improve the user experience by:

1) Improving uptime by efficiently providing troubleshooting instructions to the user

2) Reducing the need for advanced training

3) Eliminating the need for printed troubleshooting information and tribal knowledge

B-232

Influence of Light in the Bilirubin Determination

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BACKGROUNDBilirubin is a yellow substance formed in the reticuloendothelial system by degradation of old erythrocytes. Heme group from hemoglobin and other heme proteins are metabolized to bilirubin and transported to the liver where it is conjugated with glucuronic acid, before being removed by the digestive tract.According BILT3 insert Roche Diagnostics measuring total bilirubin is stable one day if the sample is between 15 and 25°C (room temperature) and 7 days when stored between 2 and 8°C (refrigerator temperature), as long as it is protected from light.The purpose of this study was to analyze the influence of ambient light and temperature of our laboratory in bilirubin degradation of samples.

METHODSThe concentration of total bilirubin in 45 samples from patients who had requested this test was determined. Then four aliquots of each sample, preserved under different conditions were performed: a) temperature and ambient light, b) darkness and ambient temperature, c) refrigerator and ambient light, d) refrigerator and darkness.Subsequently total bilirubin was determined in four aliquots at 3, 6, 9, 12 and 24 hours. This was done in 8000 COBAS analyzer Roche Diagnostics, by binding of bilirubin to a diazonium ion in acidic medium, to form azobilirubin which was measured photometrically. The obtained data were statistically analyzed by MedCalc.

RESULTSIn dark conditions, both at room temperature and in the refrigerator, the average concentration of bilirubin remained constant during the first 24 hours of receiving the sample.In ambient lighting conditions, the average concentration of bilirubin was decreasing in all samples during the first 24 hours. Decreasing the concentration of bilirubin in the samples tested, compared to baseline, was:-At 3 hours: decrease of 0.43 mg/dL at room temperature and 0.39 mg/dL in the refrigerator.-At 6 hours: decrease of 0.64 mg/dL at room temperature and 0.67 mg/dL in the refrigerator.-At 9 hours: decrease of 0.80 mg/dL at room temperature and 0.90 mg/dL in the refrigerator.-At 12 hours: decrease of 0.90 mg/dL at room temperature and 1 mg/dL in the refrigerator.-At 24 hours: decrease of 1.20 mg/dL at room temperature and 1.40 mg/dL in the refrigerator.

CONCLUSIONS

The concentration of total bilirubin remains constant during the first 24 hours if samples are stored in darkness. Under these conditions, it would be possible to extend the determination of bilirubin during this period.However, it has been observed that the ambient light of the laboratory decreases bilirubin over time. This could have clinical impact, particularly in patients whose bilirubin concentration at the time of arrival of the sample at the laboratory is pathological and with the passage of time decreases to be within our reference range (0.2 -1 mg/dL).

B-233

Optimization of Storage and Transport Temperature and Time for Complete Blood Count Testing

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Background: Reliable specimen collection and transport are fundamental to obtain high quality test results in the hematology laboratory. Recognizing the effect of fluctuations in transport and storage conditions, we performed an IRB approved laboratory experiment on 10 healthy volunteers under controlled temperature and storage conditions. This experiment concluded that complete blood count (CBC) specimens should be analyzed within 12 hours of collection and 2-8°C is the optimal temperature range for specimen storage and transport. We further performed a pilot study in two distant Patient Service Centers (sites 1 and 2) to test out the optimized storage and transport conditions.

Objective: To investigate the effects of temperature fluctuation and duration of storage and transport on CBC specimens.

Methods: All CBC specimens (n=2060) were stored at 2-8°C at each pilot site and transported at 2-8°C in courier vehicles for the month of August 2019. These CBC specimens were then analyzed within 12 hours of blood collection. MCV and MCHC data before and after implementation of the changes were compared.

Results: There was a statistically significant reduction ($p < 0.001$) in median MCV from 92.2 to 91.4 fL at site 1 and 91.4 to 90.7 fL at site 2, along with an increase in median MCHC ($p < 0.001$) from 32.5 to 33.4 g/dL at site 1 and 32.6 to 33.3 g/dL at site 2. Additionally, the percentage of high MCV reduced from 9.2 % to 6.4% at site 1 and from 7.9% to 6.3% at site 2. The percentage of low MCHC reduced from 45.6% to 20.1% at site 1 and 43.2% to 22.5% at site 2.

Conclusion: Optimizing the storage/transport temperature and minimizing time from blood collection to analysis resulted in reduction of abnormal CBC results. Our data suggested that the optimum storage and transport temperature should be 2-8°C and time from blood draw to analysis should be minimized to less than 12 hours. This project also had significant financial impacts in terms of reduction in repeat CBC, follow-up testing for anemia, and reduction in technical time to review unnecessary blood smears due to abnormal CBC results. This cost savings resulted in better patient satisfaction which is the ultimate goal of every health professional.

B-235

Cost-Effectiveness Analysis of Total Laboratory Automation in Laboratory Medicine

K. Kim. *Yonsei University, Seoul, Korea, Republic of*

Background: Automation enabled laboratory to be operated fast, precise and standardized. Many studies about positive effects on laboratory automation have emerged, but automation is still being developed and upgraded into new forms. Severance hospital, one of the biggest hospital in Republic of Korea, revamped the previous automation into new TLA(total laboratory automation) system and ended up with stabilization on Nov 2019. Automated from registration(when barcode reads the specimen arrival) to archive(conveyors connected to storage modules), TLA has been truly made. From hospital management perspective, TLA is thought to bring patient and staff satisfaction which leads to enhanced hospital experience. In this study, the overall changes from the pre-analytical to the post-analytical process are detailed to review the improved workflow of laboratory. TAT(Turn-around time), which is known as one of the key indicators evaluating laboratory performance, will be analyzed to compare the productivity. ROI(Return on Investment) will be calculated via analyzed TAT and invested cost for TLA. **Methods:** Total number of tests and average TAT of each tests were used from LIS(Laboratory Information System). 1-month LIS data for Dec 2019 was taken considering that the end of implementation and stabilization was on Nov 2019. Another 1-month LIS data for Dec 2018 was compared as a control group. For cost evaluation, the equipment cost and staff cost were used to calculate the total cost invested. **Results:** Total number of tests performed has been increased from 1,159,382 to 1,190,506 while average TAT decreased from 72.9 minutes to 68.5 minutes. The number of tests per minute was calculated by dividing total number of tests into average TAT. The number of tests per minute indicates the enhanced capability of laboratory, which has been increased 9.4%. Specifically, the capability of immunology and clinical chemistry tests have been increased 33.9% and 5.4%, respectively. **Conclusion:** The results of this study demonstrate that TLA made significant workflow changes of laboratory and brought improved productivity comparing the total amount invested.

B-236

Proposal for Practical Delta Check Criteria Based on the Real-World Data

S. Kim. *Ilsan Paik Hospital, Inje University College of Medicine, Goyang, Korea, Republic of*

Background: Although a delta check has been a widely used practice in clinical laboratories to detect errors, methods did not standardize yet. In our laboratory, we used a delta check that originated from several works of literature. We aimed to assess the utility of present delta check limits. **Methods:** A total of 121,498 paired results (82,673 for inpatients and 38,825 for outpatients) for 15 general chemistry test results from October 1, 2019, to November 31, 2019, were analyzed. the laboratory's delta values (including delta difference, delta percent, rate difference, and rate percent according to the test items) between two consecutive results in one patient were calculated among inpatients and outpatients. The detection rates of delta check for each test item were estimated. For each item, the delta criteria of one, three and five percent detection rate was presented. **Results:** When applying the laboratory's delta criteria, the detection rate of delta check of ALP, ALT, AST, BUN, and uric acid was higher than 10% for inpatients. In contrast, the detection rate of creatinine, CRP, potassium, and sodium was lower than 1% for inpatients, and the detection rate of ALP, potassium, total bilirubin, and uric acid was lower than 1% for outpatients. The detection rate was 0% for total cholesterol of inpatients and creatinine, and CRP of outpatients. Proposed delta check criteria that having one, three and five percent detection rate in inpatients were much higher than those in outpatients.

Conclusion: Inappropriate delta check criteria caused impractically high or too low detection rates. Delta check criteria based on real-world patients' results would help appropriate practice.

B-237

Simple Tool to Identify Intravenous (IV) Fluid Contamination through Glucose Measurement

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Background: Although not ideal, drawing blood samples from inpatients through intravenous (IV) catheters is a common practice, particularly in pediatric hospitals to avoid repeated sticks and in patients with limited vascular access. When drawing samples through IV catheters, fluid infusion should be completely stopped and blood volume at least three times the dead volume of the catheter should be discarded before sample collection. However, in reality, this does not happen due to incomplete clamping of the IV catheter or the inability to discard enough blood volume. Blood sample contamination with IV fluids may result in erroneous results and patient harm. **Objective**: To identify samples contaminated with IV fluids. **Material and Methods**: In our institute, we implemented several rules to identify specimens contaminated with IV fluids. These rules included specific cut-off values for certain analytes or the calculation of delta values. The analytes included glucose, sodium, potassium, chloride, calcium, ionized calcium, pH, PTT (for heparin contamination) and hemoglobin/hematocrit (for IV fluid contamination of CBC samples). Here we present data for IV fluid contaminated samples recognized though Basic Metabolic Panel (BMP) testing. The Ortho Diagnostics Vitros 5600 was used for BMP testing. **Results and Discussion**: Of all the analytes/rules studied, a glucose measurement greater than 200 mg/dL was the most effective rule in identifying IV fluid contaminated samples (295 of 41136, 0.7%). When patient floors were notified of suspected contamination and samples were recollected, 90.2% of the subsequent samples were free of contamination, demonstrating that IV fluid contamination can be avoided with reinforcement and caution. Glucose worked better than other analytes in identifying IV fluid contamination, due to a large difference between plasma and IV fluid glucose concentrations. For example, one drop (~50 µL) of D5 (5000 mg/dL) in 1 mL of whole blood, with a hematocrit of 40% will raise the plasma glucose concentration by ~400 mg/dL. This change in glucose concentration is enough to alert specimen contamination. It is important to note that the "glucose of >200 mg/dL" rule was not applied to specimens with CO_2 less than 12 mmoles/L in order to avoid a delay in reporting high glucose samples from diabetic patients who generally have low CO_2. Although the study did not cover other laboratory areas such as coagulation, hematology, and therapeutic drug monitoring, identifying IV fluid contamination in one area can be used as a broad teaching tool for correct sample collection. We have used this as a tool in reducing IV fluid sample contamination at our institute and have reduced the IV fluid contamination by ~30%. **Conclusion**: Glucose monitoring is an effective way of identifying IV fluid contaminated samples.

B-238

Use of a Lean Six Sigma Approach to Investigate Excessive Quality Control Material Use and Resulting Costs

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Background: To analyze the procurement costs of quality control materials and develop an Individualized Quality Control Plan (IQCP) using Lean Six Sigma. Eastern Health routine Clinical Biochemistry Laboratory is a highly organized, ISO 15189 plus accredited lab catering to the province of Newfoundland and Labrador. All routine clinical chemistry and immunoassay tests are done on Abbott Architect systems. This laboratory functions as a referral facility for smaller sites within the province. Significant increases in costs for quality control (QC) materials were noted over the course of a five year QC supplies contract so an analysis of QC practices was initiated. **Methods**: A project charter was developed based on DMAIC process and a six sigma team was constituted. Regular Starburst Brainstorming sessions were organized to discuss the project plan. Data was collected on sources of preventable wastage and areas for improvement and potential cost savings. A variety of six sigma tools were used during planning, for review and data analysis. A risk assessment was done for proposed changes in procedures and to evaluate impact of changes in QC processes that were redesigned based on input from the team based data analysis. **Results:** Eastern Health is investing approximately CAD 400,000 annually in purchase of QC materials for routine clinical chemistry and immunoassay. Review of quality control processes showed several defects in processes including non-optimized use of auto analyzers; programming of several low workload tests on more than one analyzer; performance of routine low workload tests on multiple sites; and a QC frequency based on two repeats of each level over the day for all tests. The ratio of QC test to patient test were as high as 10 to 100 for some urine chemistries, and 5 to 10 for some therapeutic drug assays. Furthermore, the volume of QC material poured into measurement vials greatly exceeded that needed to complete tests, and there was an unexpected high number of calibrations for these chemistries. In response specific corrective actions were directed against each driver of excessive QC material use and Individualized Quality Control plans were developed based for tests **Conclusions**: Use of Lean six sigma strategies and tools provide an effective means for identifying drivers for QC material use for targeting corrective actions to reduce wastage of QC materials and the associated costs.

B-239

Performance Evaluation of the Atellica CH HIL Feature

J. T. Snyder, E. Garcia, J. Davis, J. Cheek. *Siemens Healthineers, Newark, DE*

Background: The Atellica® CH Analyzer offers 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 following assays: ALT, ALTPLc, AST, ASTPLc, LDLP, and 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. H and L indices were assigned based on the expected concentrations. I indices were assigned using the Atellica® CH TBil_2 Assay. Hemolyzed, icteric, and lipemic samples targeting each of the 0 to 6 Atellica CH HIL indices, and samples with no visible hemolysis, icterus, and lipemia, were processed with n = 3 replicates with each HIL technique. Mean observed results were compared to the expected results. **Results:** The results are summarized for HIL results produced using all six techniques described above. For hemolysis, the Atellica CH Analyzer correctly matched the expected values with added hemolysis at 0, 70, 190, 375, 625, 875, and 1200 mg/dL hemolysis and with 20 visibly clear serum and lithium heparin plasma samples. For icterus, the Atellica CH Analyzer correctly matched the expected values with added conjugated bilirubin at <0.1, 5, 16, 26, 34, 44, and 60 mg/dL conjugated bilirubin in 33 out of the 42 opportunities and with 20 visibly clear serum and lithium heparin plasma samples. For the 26 mg/dL conjugated bilirubin sample, the HIL techniques produced observed indices ranging from 3 to 4 with the expected I index of 3. With the 34 mg/dL conjugated bilirubin sample, the HIL techniques produced observed indices ranging from 4 to 5 with the expected I index of 4. All observed icterus results were within ±1 index of the expected value. For lipemia, the Atellica CH Analyzer correctly matched the expected values with added INTRALIPID at 0, 200, 375, 600, 850, 2000, and 3500 mg/dL INTRALIPID and with 20 visibly clear serum and lithium heparin plasma samples. **Conclusions:** The Atellica CH Analyzer HIL output produced by the ALT, ALTPLc, AST, ASTPLc, LDLP, and stand-alone HIL tests all agreed with the expected value within ±1 index unit.

Product availability varies by country and is subject to regulatory requirements.

B-240

Performance Evaluation of Sample Carryover on the Atellica CH Analyzer

J. T. Snyder, J. Kollhoffer, J. Cheek. *Siemens Healthineers, Newark, DE*

Background: On integrated systems that include both immunoassay and clinical chemistry analyzers, sample carryover can arise when analyte adsorbs to a reusable probe (inside, outside, or both) and contaminates a subsequent sample or cuvette in the testing sequence of the analyzer. Incomplete washing of the sample aspiration/dispensing device can cause this type of carryover. This study evaluated the potential for sample carryover on the Atellica® CH Analyzer by means of its dilution probe and sample probe. **Methods:** Two analytes were selected for measuring potential carryover: cannabinoids (THC) and hepatitis B surface antigen (HBsAg). The impact of high (positive) samples run immediately before low (negative) samples was assessed. For THC, samples were analyzed on the Atellica CH Analyzer. For HBsAg, the samples were first submitted to the Atellica CH Analyzer to process an ALT test and then analyzed on an ADVIA Centaur® XP Immunoassay System. The concentration of the high-positive samples was 1070 ng/mL for THC and 2.98 mg/mL for HBsAg. **Results:** THC: The results for all the negative THC urine samples were below the measuring interval (<15 ng/mL) for all tests, regardless of whether or not they were preceded by a high THC sample. HBsAg: The results for all the negative HBsAg serum samples were below the measuring interval (<0.1 Index Value) for all tests, regardless of whether or not they were preceded by a high HBsAg sample. **Conclusions:** In these experiments, when using high-positive samples at concentrations at or greater than the upper limit of the clinical range, no detectable levels of THC and HBsAg sample carryover were found for the Atellica CH Analyzer.

B-242

Not T Too! False Elevations in High-Sensitivity Cardiac Troponin T (hs-TnT) following Transportation of Specimens between Testing Sites

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Introduction: Pre-analytical false elevations attributed to fibrin strands in plasma have been reported for Troponin I but not Troponin T (TnT). Here, we describe reproducible false elevations in high sensitivity TnT (hs-TnT) in specimens that have been transferred between collection and testing sites in their original Lithium-Heparin plasma separator tube (Lihep-PST). **Methods:** Five pre-analytical conditions were tested for patient specimens collected in a Lihep-PST and assayed for hs-TnT (Roche Cobas e602). 1) Collected and tested at one location (N=24). 2) Collected offsite, centrifuged, and transported STAT <2hrs (N=69) or, 3) ROUTINE >2hrs (N=66). 4) Collected offsite, centrifuged, and aliquoted prior to transport (N=87). On arrival, Lihep-PST samples collected under conditions #1-4 were assayed for hs-TnT, an aliquot re-centrifuged, any potential residual fibrin removed using a wood applicator, and then re-measured. 5) Collected offsite from testing location, centrifuged, and transported STAT <2hrs, and hs-TnT measured as in previous experiments (N=16), but samples discrepant for hs-TnT between the Lihep-PST and an aliquot were kept at room temperature upright for 24 hours and assayed for hs-TnT at 1hr, 2hrs, 4hrs, and 20-24hrs (N=6). In all experiments, a difference of 5ng/L if hs-TnT #50ng/L or 10% if hs-TnT >50ng/L was considered significant. In order to isolate the interfering substance and confirm fibrin as the cause of the false elevations, plasma pools discrepant for hs-TnT underwent 3 successive rounds of gentle centrifugation (1min at 55xg), removal of the supernatant, and replacement of the supernatant with Roche MultiAssay diluent (N=3 pools). The final washed resuspension thought to contain fibrin was assayed for hs-TnT. **Results:** 30 of 69 (43%) samples collected under pre-analytical condition #2 (Lihep-PST collected offsite, centrifuged, and transported STAT <2hrs) were found to have clinically relevant decreases in hs-TnT between initial and re-centrifuged measurements. The absolute decrease in hs-TnT was significantly higher in discrepant (median=9.9ng/L, 95% CI 7.5-13.9ng/L) compared to non-discrepant (median=2.8ng/L, 95% CI 1.0-3.8ng/L) samples (p<0.0001, Mann Whitney test). None of the samples from experiments #1, 3 or 4 had clinically significant differences

in hs-TnT results between initial and aliquoted measurements. When Lihep-PSTs identified as discrepant (N=6) were left upright at room temperature, hs-TnTs were on average 53, 45, 31, and 4% of their absolute discrepancies at 1hr, 2hrs, 4hrs, and 20-24hrs, but were no longer significantly different from rimmed aliquots by 4 hrs (p=0.61), repeated measures ANOVA with Dunn's multiple comparisons). Attempts to reproduce the false elevations using fibrin alone from washed samples were not successful. **Conclusion:** Here, we report reproducible pre-analytical false elevation in hs-TnT following STAT inter-site transport of specimens. False elevations occurred in 40% of separated Lihep-PST tubes transported and measured in <2hrs. These false elevations are clinically significant in the 3-14 ng/L range used in rule-in vs rule out algorithms for Myocardial Infarction. Interference does not occur if plasma is aliquoted or if hs-TnT is tested at the collection site. All samples for hs-TnT measurement, requiring transport between sites, should be aliquoted at the collection location prior to transport.

B-243

The Effect of Aerosol Contamination in A Chemistry Analyzer

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Background: One Siemens ADVIA-2400 was introduced to Emergency Laboratory (2400-EL) with Aptio Automation. During performance verification, LIS (HopeBridge) suggested that LDH results were highly unstable. A comprehensive investigation was initiated to resolve the problem.

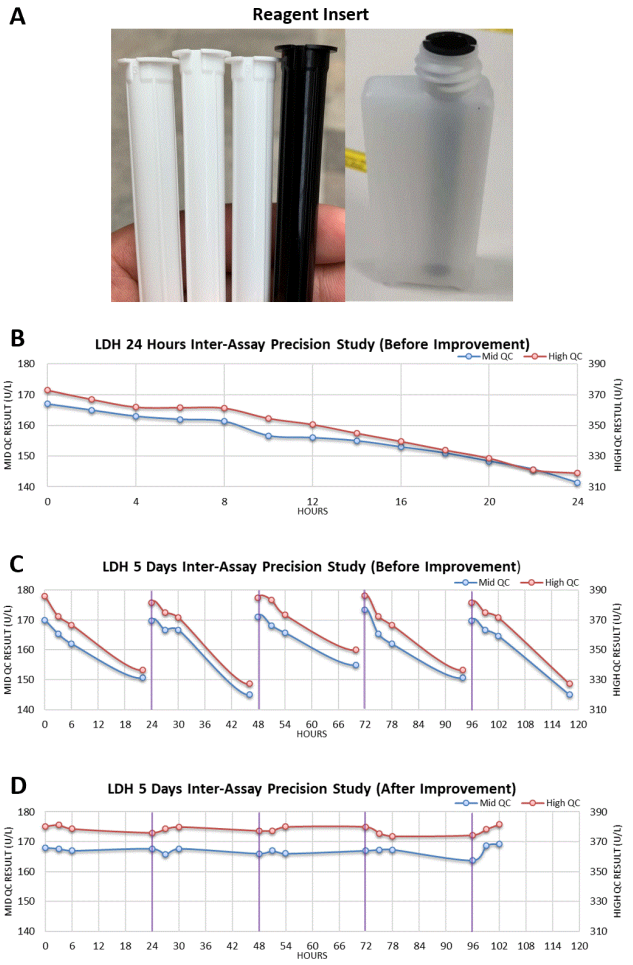
Method: LDH reagent (Siemens) and QC sample (BIO-RAD) causes were ruled out as tests on other two ADVIA-2400 analyzers in the Central Laboratory were constantly under control. The hardware was tested thoroughly, and the programme was updated to prevent reagent probe contamination. However, reagent suffered severe decay after 5 days without any testing (mid QC from 168 to 66; high QC from 376 to 152) while suggested stability was 30 days. After eliminating all other possibilities, aerosol contamination was suspected.

Comparing with other analyzers, 2400-EL had four extra assays (CHE, Fe, TRF and UIBC, Merit Choice Bioengineering). Each assay was paired with LDH for: intra-assay precision (n=20), 24-hour inter-assay precision (1-batch/2hrs, 3-run/batch, n=39), and 5-day inter-assay precision with fresh LDH reagent everyday (4-batch/day, 3-run/batch, n=60). Precision studies were assessed using mid and high QC samples.

After using Siemens Reagent Insert (Figure A) in R_2 reagents to prevent reagent decay, intra-assay and 5-day inter-assay precision (3-batch/day, 3-run/batch, n=45) studies were assessed.

Results: Study indicated that LDH was impacted by UIBC reagent. The mid and high %CV for LDH precision studies were, respectively, as follows: 1.30% and 1.23% (intra-assay); 5.04% and 5.14% (24hrs inter-assay), 5.34% and 5.37% (5d inter-assay). Both inter-assay precision %CVs were greater than 1/3 TEA (3.67%) which failed the performance verification. With Reagent Insert, the mid and high %CV for precision studies were: 0.86% and 0.84% (intra-assay); 1.07% and 0.95% (5d inter-assay), respectively. Detailed QC results show in Figure B-D.

Conclusions: Aerosol contamination in analyzer should be closely monitored when reagents from multiple providers are used, and Reagent Insert could improve reagent stability significantly when such issue occurs.



B-245

Phlebotomy Device Preference and Specimen Quality in an Oncology Outpatient Clinic: Comparison of BD UltraTouch™ Blood Collection System and BD Vacutainer Push Button Blood Collection Set

V. D. Merrill, M. D. Ward, D. Anderson, J. McNeil, E. Pickett, S. Duh, R. H. Christenson. *University of Maryland School of Medicine, Baltimore, MD*

Background: Oncology patients have frequent venipuncture which causes scarring that makes subsequent draws difficult and painful. Novel blood collection systems provide an opportunity to decrease discomfort in patient populations having repeat blood draws. The UltraTouch™ was developed for this purpose; the outside design has diameter of the needle is 25 gauge but the lumen is equivalent to a 23 gauge device.

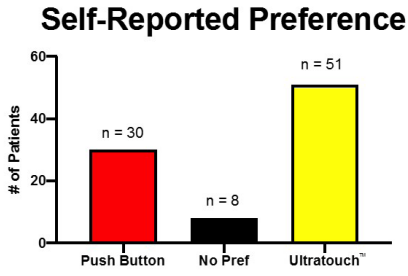
Purpose: The objective of this study was (1) to determine whether the UltraTouch phlebotomy system is preferred by patients over the current Push Button (PB) system and (2) to assess specimen quality by comparative measures of plasma and serum analytes.

Methods: We recruited subjects from an oncology outpatient clinic to determine their preference for either the standard PB or the UltraTouch blood collection system (both from BD, Franklin Lakes, NJ). By design, subjects received two blood draws, one with each device, one for each arm. Device used first was randomized and subjects were blinded to device identity. The same phlebotomist collected all samples. Specimen quality was assessed for each device with measurements for plasma hemoglobin (Shimadzu UV-1800), and serum lactate dehydrogenase and potassium (Vitros 4600/5600).

Results: Of 101 participants, 12 required second-stick in one/both arms and were excluded from analysis. Data for the remaining 89 participants are shown (Figure). Preference for the UltraTouch over the PB device was significant (p = 0.0196). In a

non-inferiority analysis, i.e. subjects favoring UltraTouch or having no preference versus PB was also significant (p = 0.0021). Regarding sample quality, UltraTouch-drawn samples had significantly lower plasma hemoglobin, average 9.37, vs. PB at 5.34 mg/dL (p<0.0001); LD and K difference was not significant.

Conclusion: Patients in an oncology clinic preferred phlebotomy with the UltraTouch system compared to the PB system in this randomized study. Also, UltraTouch-drawn samples had less hemolysis as assessed by plasma Hb.



Non-inferiority analysis
Chi-squared results for
(59 vs 30), *p = 0.0021*

UltraTouch™ vs. Push Button
Chi-squared results for
(51 vs 30), p = 0.0196

B-246

Impact of Automation and Monitoring on Turn Around Time of STAT Tests

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Background:Laboratories round the globe are increasingly adopting Total Laboratory Automation (TLA). The major goal of automation is to increase efficiency and productivity. Turnaround time (TAT) is used as one of the main quality indicators to monitor these parameters. TLA is aimed to make processes lean, eliminate redundant steps and minimize manual inputs to achieve high throughput and error free performance. Our objective was to study the impact of automation and monitoring on Turnaround Times of Stat Tests.Material and Methods:Turn-around times of stat tests ordered from the Emergency Department (ED) were analyzed for three months before and fourteen months after adopting total laboratory automation on Abbott ACCELERATOR a3600 in a tertiary care hospital based laboratory in Karachi, Pakistan. The processes used prior to automation required manually centrifugating the specimens and analyzing on a variety of analyzers, namely two Roche cobas c311, Rx Imola, Roche cobas e411, two Vitros Eci, and two Nova CRT- 4, followed by stacking and storing the specimens in different refrigerators and room temperature units. Automation on Abbott a3600 track aligned the process to an input output module, two pre-analytic centrifuge units, two Alinity ci analyzers as well as a post analytic refrigerated storage, with Analyzer Management System (AMS) as middleware. Benchmark was set at one hour after specimen receiving. The tests included in STAT list were glucose, urea, creatinine, electrolytes, amylase, total and direct bilirubin, calcium, magnesium and troponin I. Gel separator tubes were changed to lithium heparin tubes for the ED to serve as an identifier as well as eliminate clotting requirement before centrifugation. Results:Turnaround time targets were maintained at 94% with 6% delay before adopting automation. The transition phase saw a huge rise in the reporting delays with delay percentages climbing up to 14%. This was then controlled in the preceding months and steadily decreased to a level slightly lower than pre automation stage. A second sharp fall in turnaround times was observed exactly a year after TLA installation by establishment of a Quality Assurance unit in the section with staff sensitization and regular, conscious and systematic monitoring of the delays. With these steps, the achieved turnaround time of STAT tests at one hour benchmark was greater than 99%. Conclusion:Total laboratory automation optimization along with conscious monitoring of the work flow can dramatically reduce total turnaround times of stat tests.

B-247

Evaluation of Veravas VeraPrep Biotin™: A Useful Tool for Investigating Immunoassay Interference from Biotin

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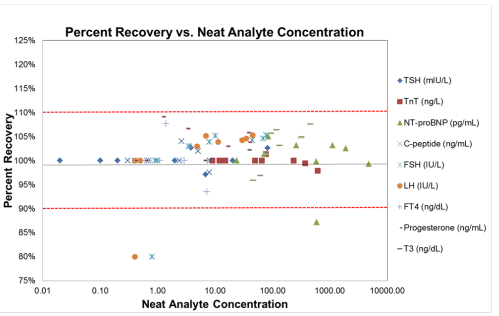
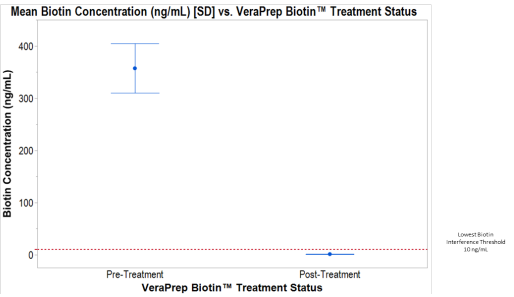
Background: Many immunoassays are susceptible to interference from biotin if patients ingest doses found in over-the-counter hair, skin, and nail supplements. It is important for clinical laboratories to have protocols for investigating biotin interference. Veravas developed VeraPrep Biotin™ to rapidly deplete biotin from clinical serum/plasma samples. **Objectives:** The aims of this study were to verify that VeraPrep Biotin™ (VeraPrep) reagent: 1) does not impact results of Roche immunoassays in control (no biotin) samples, 2) can effectively deplete biotin spiked/added into serum samples, and 3) can effectively deplete endogenous biotin in samples from donors who ingested biotin supplements. **Methods:** De-identified residual waste serum/plasma samples were combined to create 9 pools for each immunoassay. Plasma samples were obtained from 6 healthy donors at varying times (n=23) following ingestion of biotin supplements (20, 100, or 200 mg). The following Elecsys assays were evaluated using the e602 (Roche Diagnostics, Indianapolis, IN): C-peptide, FT4 II, follicle stimulating hormone (FSH), luteinizing hormone (LH), N-terminal pro-brain natriuretic peptide (proBNP), progesterone III, T3, thyroid stimulating hormone (TSH), Troponin T Gen 5 STAT (TnT). Control, biotin-spiked (n=10, ~400 ng/mL), and donor samples were assayed pre- and post-VeraPrep treatment according to manufacturer's instructions. Percent analyte recovery ((post-treatment/pre-treatment)*100) was calculated for control samples. A lab-developed LC-MS/MS method was used to quantify biotin. **Results:** In control samples (n=81), the median(interquartile range (IQR)) analyte recovery post-VeraPrep treatment was 100(100-103)% (Figure 1A). The mean±standard deviation [spiked-biotin] was 357±47 ng/mL and 1.0±0.6 ng/mL pre- and post-VeraPrep treatment, respectively (Figure 1B). The median(IQR) [biotin] in donor samples was 73(32-147) ng/mL and <0.1(<0.1-0.1) ng/mL pre- and post-VeraPrep treatment, respectively.

1A



1B



Conclusions: These data demonstrate that VeraPrep treatment does not affect analyte recovery in biotin-negative samples and effectively depletes both spiked and endogenous biotin in serum/plasma. VeraPrep Biotin™ is a useful tool for the investigation of biotin interference in clinical laboratories.

B-248

Variation in Vitamin C Dosage With and Without the Use of a Refrigerated Centrifuge and after Thawing the Sample

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Background: The measurement of vitamin C involves pre-analytical care which can keep the vitamin stable until its analysis. Due to its unstable nature and photosensitivity, routine vitamin C laboratory dosing requires that certain parameters be followed. Therefore, establishing the best sample (plasma or serum), the use or not of preservative solutions and refrigerated centrifuge after sample collection, protection from light and freezing of the sample are key points for its stability. **Objective:** To evaluate the need to use a refrigerated centrifuge (4º C) for centrifuging the sample for dosage of Vitamin C, as well as its stability after thawing.

Methods: Twenty samples from patients of both genders, with or without vitamin C replacement, were obtained. The serum samples were protected from light from collection to analysis by High Performance Liquid Chromatography (HPLC). After the analysis, the sera were again frozen and thawed on the fourth day after the first dosage of vitamin C. **Results:** Inaccuracy studies were performed using Pearson's Correlation and T-tests (difference between means) for the two hypotheses tested, that is, difference between samples centrifuged in refrigerated and non-refrigerated centrifuges and difference between the results before and after thawing. Regarding the use of refrigerated and non-refrigerated centrifuges, we observed a correlation between samples (r = 0.998), with no difference between the means, T-test 0.26, less than the T critical of 2.06. Regarding vitamin C results before and after thawing, we obtained a negative correlation between the results (r = 0.996), intersection -0.809. Moreover, there was a difference between the means, T-test = 6.99 above the T Critical = 2.09 **Conclusion:** The absence of centrifugation in a refrigerated centrifuge does not interfere with the analysis of Vitamin C, but there is a significant loss of stability after thawing the sample.

B-249

Use of Lean Management to Increase Productivity of Procalcitonin Testing for Sepsis Management

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Background: Throughout Henry Ford Health System, procalcitonin (PCT) testing is a part of a system-wide initiative for managing sepsis patients with the goals of enhanced patient safety, earlier decision making, shortened ICU stay, earlier discharge, and fiscal savings. PCT testing in the automated core laboratory is currently performed on an offline analyzer (Roche Cobas) in batch production. The objectives of this study were to determine if migrating PCT testing to a newly released PCT assay (Beckman Access PCT) on a high-throughput analyzer on a Beckman Coulter automation line could increase productivity facilitated by one-touch continuous flow, reduce motion and time waste, and create 24/7 STAT testing options while providing the high level of assay performance needed to support sepsis treatment decisions.

Methods: Baseline turnaround times (TATs) for the existing offline PCT assay were collected from the laboratory information system (LIS). The current testing strategy requires moving specimens from a Beckman automation line outlet sorting station to the offline workstation. Because PCT is currently not tested 24/7 but is batched for staffing reasons, we expected a wide range of TATs in our offline testing scheme. The periods from receive-to-result (received by the automation system inlet to result posted to LIS) were measured. Value stream maps were constructed to calculate lead time and identify areas of waste to quantitate potential efficiencies gained by using automation versus manual worker intervention. Analytical assessment of the new Access PCT assay included linearity, imprecision, detection capability (LoB, LoD, LoQ), and accuracy compared to the Cobas e411 PCT assay.

Results: A total of 2400 PCT results were captured over the course of 10 weeks on the two workflows. Productivity studies favored the online automated system, with TATs of 34.1/30.2 (mean, median) minutes compared to 224.9/176.0 for the offline workflow where the shortest TAT observed was 52 minutes. The automated scheme provided more consistent TATs, with a standard deviation of 8.4 minutes compared to 120.3 minutes for the non-automated workflow. In addition, motion waste was reduced by 62.1%, manual touches reduced by 71.1%, and hand-offs between staff by 75.0% using the automated system. These efficiencies could produce savings in both time (232.8 minutes) and motion waste (2256 steps) per week. In analytical validation studies, the Access PCT assay performed favorably with total imprecision ranging from 3.59 to 3.68% CV. LoB, LoD, and LoQ for the investigational Access assay were quite acceptable, at 0. 0.00053, 0.00097, and 0.0019 ng/mL, respectively. Method comparison yielded a Deming regression equation of Access = 1.114 × Cobas + 0.115 ng/mL, r^2 = 0.9857. Analytical concordance analysis yielded 100% agreement to Cobas at 0.25 ng/mL.

Conclusion: Migrating PCT testing to an online automation-based immunoassay system resulted in equivalent analytical performance to our off-line system but achieved faster and more consistent turnaround times with less human intervention. On an annual basis, these enhancements in productivity would eliminate 117,495 steps (55.6 miles) and reduce labor waste by 201 hours, allowing increased testing capacity and enabling staff to be redirected to other tasks.

B-250

Utilization of Vitamin B_{12} Tests in a Major Reference Laboratory Suggests a Lack of Evidence-Based Rrdering Practices by Community Physicians

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Objective: To assess the evidence-based utilization of vitamin B_{12} testing in a large reference laboratory. **Introduction:** Vitamin B_{12} is an enzyme cofactor for both methionine synthase and methylmalonyl-CoA mutase required for reactions in DNA, protein and myelin synthesis. Megaloblastic anemia due to interrupted red blood cell DNA synthesis and neurological changes including cognitive deficit and paresthesia are the primary clinical manifestations of vitamin B_{12} deficiency. At-risk populations include the elderly, strict vegans, alcoholics, and patients with pernicious anemia, intestinal malabsorption, gastric or intestinal surgery. In our laboratory, B_{12} is ordered at very high numbers (>280,000 tests per year; population 1.28 million), despite the fact that deficiency is rare due to fortification of food and the availability of these vitamins in the diet. **Methods:** De-identified patient results from venous blood samples for the period of September 01, 2018 to August 31, 2019 were obtained from the Laboratory Information System. Specific test results queried included vitamin B_{12}, folate, CBC parameters including mean corpuscular volume (MCV) and creatinine. Matched homocysteine and methylmalonic acid results ordered within 1 week of a B_{12} order were also obtained. Patient age, collection location, and ordering physician speciality were also collected. Data was analyzed in Excel v.1912. Differences between groups were analyzed using Chi-square test of independence. Between group significance was set at $p<0.05$. **Results:** Our institution performed 282,683 vitamin B_{12} measurements on patients over 10y old during a 1-year period. 12.1% of every specimen with a serum creatinine and 12.1% of every specimen with a CBC performed at our institution also had a B_{12} measurement ordered. 2.5% of all vitamin B_{12} results were below our current reference interval (RI; 155-700pmol/L). 80.3% of all vitamin B_{12} measurements had a matched MCV, of which only 1.8% indicated macrocytosis (MCV >100fL); 3.2% of vitamin B_{12} measurements below the RI had an associated macrocytosis. Additional biomarkers that are recommended in the clinical investigation of conditions associated with vitamin B_{12} deficiency (folate, homocysteine and methylmalonic acid) were only ordered together with a B_{12}, 9.3, 2.4 and 0.4% of the time, respectively. 89.6% of all B_{12} tests were ordered by community family physicians, only 2.4% of measurements were below the RI. 10.0% of all B_{12} tests were a repeat measurement on the same patient. One patient received 19 measurements with each value within the RI. 61% of all B_{12} tests were ordered on females. The prevalence of vitamin B_{12} deficiency did not differ between inpatient (2.4%), outpatient (2.5%) or aged care facility (2.3%) populations (X^2=2.75, p=0.25). There was no significant difference in the prevalence of B_{12} deficiency in patients under 60y old (2.3%) compared to over-60y olds (3.3%) (X^2=0.78, p=0.94). The prevalence of vitamin B_{12} deficiency also did not differ according to medical specialties ordering the test. **Conclusions:** In our institution, 90% of vitamin B_{12} tests are ordered by community family physicians, indicate sufficient levels in 97.5% of patients, and do not follow an ordering pattern suggestive of investigating vitamin B_{12} deficiency. The lack of evidence-based vitamin B_{12} testing should be stopped.

B-251

Survey of Practices for Performance of Urinary Albumin in Laboratories from Ontario and Newfoundland

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Objective: To evaluate laboratory practices for the ordering, analysis and reporting of urinary albumin (UA) among participants enrolled in the routine urine chemistry survey from the Institute of Quality Management in Healthcare (IQMH), which provides ISO 17043:2010 accredited Proficiency Testing (PT) programs for medical laboratories. The scope included test nomenclature, specimen documentation, decision thresholds, and result interpretation. **Methods**: Briefly, the following questions were included with the IQMH Urine Chemistry PT survey in August 2019: 1) What is the name of the UA test? 2) Which test(s) are offered for assessment of albuminuria? 3) How does the laboratory document the collection time for random urine samples when done outside of the collection laboratory? 4) How does the laboratory handle results for albumin-creatinine ratio (ACR) when UA levels are above the measurement range? 5) What is the normal range used for interpretation of urinary ACR and 24-hr UA? 6) What is the source of normal range(s) used for ACR? **Results:** One hundred seventeen laboratories in Ontario and Newfoundland received the survey. Of those, 77 responses were included in data analysis of the pre-analytical questions, while 52 were included in data analysis of the analytical and post-analytical questions. The majority (70%) reported use of the term 'microalbumin', while 30% use the term 'urine albumin' on the test menus and reports. Forty-eight laboratories (62%) offer both random and 24-hr (timed) UA, and only 30 laboratories (39%) offer both ACR and PCR (protein-to-creatinine ratio) for assessment of albuminuria. Fifty-six laboratories (73%) indicated that they offer either ACR or PCR in addition to reagent strip urinalysis for protein (dipstick). Urine collection time is self-reported by patients according to 60 laboratories (86%). For testing laboratories, 65% indicated that they dilute samples with high UA results and report actual ACR, while practices varied in the remainder that do not dilute. Laboratories reported normal cut-offs for ACR in males and females that ranged from <1.5 to <3.5 mg/mmol creatinine (<15 to <35 mg/g Cr). The largest group (50%), used <2 mg/mmol (<20 mg/g Cr) for males consistent with the 2018 Diabetes Canada clinical guidelines, although the largest group for females (27%) used a cut off of <2.8 mg/mmol (<28 mg/g Cr) from the previous guideline. Other reported sources included manufacturer's package insert (21%), American Diabetes Association (ADA) guidelines (8%), Canadian Society of Nephrology (6%), Kidney Disease Improving Global Outcomes (KDIGO) 2012 (4%), the UK National Institute for Health and Care Excellence (NICE) 2014 (2%) and other sources (15%), some of which differ in their thresholds for albuminuria.

Conclusions: Considerable variability in responses to all of the survey questions demonstrates a need for standardization of naming, testing, reporting, and interpretation of albuminuria across those surveyed. The lack of unified recommendations by national and international clinical guidelines may contribute to inconsistent practices that impact on patient care.

B-253

Evaluation of the PAAN™ of 3 Different POC Lactate Assays (PreAnalytic Variation, including Biologic and ANalytic Variation) Demonstrates Acceptable Performance in Patients with Non-Elevated Lactates

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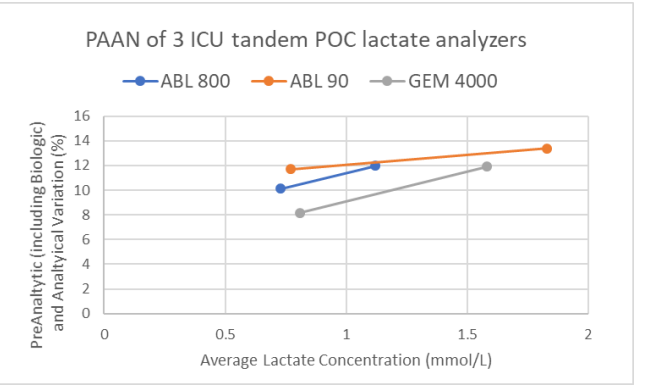
Background: When compared to a previous measurement, all clinical laboratory measurements exhibit 3 different variations, preanalytic, biologic (BV) and analytic. We can derive this variation by assembling large numbers of intra-patient test values and calculating the intra-patient variation for increasing time intervals between the repeated tests (Cembrowski). Over short periods, the patient variation vs time is linear as it represents a Taylor's series of an exponential function and the y intercept provides the magnitude of the mixture of variations. We call this y intercept value PAAN™. As lactate is not a primary component of many machine learning (ML) sepsis algorithms, we speculated that lactate's analytic variation might be too high for ML inclusion. We evaluated PAAN™ in 3 popular POC assays, the IL GEM 4000, the Radiometer ABL800 and ABL90. **Methods:** We obtained de-identified safePICO blood gas syringe-drawn arterial lactates and their analysis date/time from the University of Alberta Hospital General Systems ICU from Sept 2017 to August 2019 (48,000 lactates), Ottawa General ICU, from Sept 2016 to Jan 2020 (17,500 lactates) and Calgary Foothills adult ICU from Sept 2017-August 2019 (21,000 lactates). We generated graphs of the variation of the three methods and two patient subpopulations: low lactates (1P to 25P) and usual lactates (25P to 75P). PAAN™ was divided by the mean lactate of each population to provide relative standard deviations. To assess the test's analytic robustness, the normalized PAANs™ were divided into the BV of 20.3%. **Results:** The Figure compares the normalized PAAN™ levels with the GEM 4000 being somewhat lower. The analytical robustness quotients, PAAN™/BV, range from 1.6 to 2, yielding desirable measures of analytical goodness. **Conclusion:** We recommend that sepsis ML include analytically desirable lactates. Serial lactates should not be interchanged if they are produced by dissimilar analyzers.



B-254

Establishment of Concentration-Specific Hemolysis Interference Thresholds for the Elecsys Troponin T Gen 5 STAT Assay as a Means to Reduce Specimen Rejection and Recollection Rates

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Background Accurate Troponin T (TnT) results are critical for the diagnosis of myocardial infarction. Hemolysis is known to cause a false decrease in results using the TnT Gen 5 STAT assay (Roche Diagnostics, Inc.). In our practice, we reject and recollect samples submitted for TnT testing when the hemolysis index (HI) exceeds the manufacturer's recommended hemolysis limit (HI=100, ~100 mg/dL free hemoglobin). This practice can lead to delays in patient care. Therefore, we evaluated the impact that hemolysis has on TnT results with the goal to establish concentration-specific hemolysis interference thresholds and thus reduce specimen rejection rates without compromising the accuracy of results. Methods Residual specimens from physician-ordered TnT were used to prepare four plasma pools with the following TnT concentration ranges: 6-15, 20-60, 100-150, and 190-240 ng/L. Hemolysate was prepared from two residual lithium heparin samples that were pooled, frozen, centrifuged, and then analyzed on a Roche cobas c501 (Roche Diagnostics, Indianapolis, IN) to obtain the baseline HI. Hemolysate was added to the plasma pools to obtain HI ranging from 0-350. Hemolysate constituted <10% sample volume to maintain sample matrix integrity. TnT was measured in duplicate on a Roche cobas e411 (e411), e601, and e602 (e601/e602). HI values were obtained from c501. TnT results in samples with increasing hemolysis were compared to the reference sample with no hemolysate. The absolute and percent bias with increasing HI was calculated. Recovery within ±4 ng/L or ±8% of initial TnT result was considered acceptable. Retrospective data from physician-ordered TnT tests performed between 7/2018-6/2019 in the Hospital Clinical Laboratory at Mayo Clinic, Rochester, MN were obtained. The rejection/recollection rate was calculated using both the manufacturer's recommended limits and the newly established concentration-specific HI thresholds. McNemar's test was used to compare the recollection rates. Results Acceptable hemolysis interference thresholds were established for TnT <6-35, 36-240, and >240 ng/L at HIs of 175, 160, and 275, respectively. During the timeframe reviewed, there were 39,063 orders for TnT in which 240 samples (0.6%) were rejected and recollected based on the manufacturer's recommended hemolysis limit (HI<100 for all concentrations). By applying these new concentration-specific hemolysis thresholds, 142 specimens (0.4%) would have been rejected due to hemolysis, thus eliminating recollection of 98 specimens (41% reduction). The difference in recollection rates was statistically

significant ($p<0.0001$). Conclusions By establishing concentration-specific hemolysis interference thresholds for the 5th Gen TnT assay, the laboratory could have reported 41% more TnT results without requiring sample recollection or compromising the accuracy of results. This process improvement may have additional downstream benefits such as cost savings associated with not having to recollect patient specimens, faster turnaround for time-sensitive results, improved patient/provider satisfaction, and better utilization of phlebotomy and laboratory resources.

B-255

The Importance of Confirming Low Levels of Vitamin C due to Various Factors that could Change that Outcome

J. D. Souza, P. B. M. C. Araujo, A. C. Bispo, L. S. Santos, D. S. Rodrigues, L. H. Hasselmann, A. F. D. F. Perozo, Y. Schrank, M. F. M. C. Pinheiro, D. M. V. Gomes, M. D. C. Freire, G. A. Campana, R. G. Fontes. *DASA, RJ, Brazil, Rio de Janeiro, Brazil*

Background: Vitamin C (ascorbic acid) is a water-soluble vitamin, which is extremely unstable and reacts with oxygen in the air, with light and with water. When exposed, chemical reactions occur that are capable of destroying it. Therefore, routine vitamin C laboratory dosing requires that certain parameters be followed such as the use of refrigerated centrifuge after sample collection, protection from light and freezing of the sample, to avoid instability of the sample leading to false low results. The aim of this study was to assess the percentage of patients that were summoned for a second collection of vitamin C due to a result under the lowest limit of detection of the method and to assess if the second result was compatible with the first obtained. **Methods:** Evaluation of anonymously data of vitamin C measurements carried out in a private laboratory from Rio de Janeiro, Brazil, from October to December of 2019. Within this time interval, every sample with the result of less than 0.4 mg/L (detection limit), that lead to a request of a second sample for confirmation according to the standard laboratory procedure, was recorded, as well as vitamin C data from patients that actually returned for the second collection. The laboratory follows pre-analytical steps as follows: collection in an amber tube, clot retraction, followed by centrifugation and freezing. Vitamin C analyses is performed by High Performance Liquid Chromatography (HPLC, Agilent Technologies Infinity), and the reference value of normality is 4.6 to 15 mg/L. **Results:** A total of 18,593 vitamin C measurements were carried out in our laboratory during this time interval. Of this total, 844 (4.54%) patients were recalled for the result confirmation, due to a result of less than 0.4 mg/L. Of the total number of recalled patients, 580 (68.72%) returned for a second collection. From those patients, 160 (27.59%) were inside the reference value, 27 (4.65%) were above the reference, and 393 (67.7%) were below the reference [of those, 127 (21.9%) confirmed the result of less than 0.4 mg/L]. **Conclusion:** According to the results obtained, we see that the majority of patients who had a new collection for vitamin C measurement confirmed results below the reference, but not as low as the results presented in the first sample. Thus, 32.1% of the patients that needed a second sample collection because presented very low results in the first sample, had some interference with the measurement. Because several factors can influence serum vitamin C levels such as age, sex, smoking, serum lipids, body weight and dietary iron, it is crucial to repeat the exam before releasing low result, although pre-analytical factors can not be excluded.

B-256

Metrological Traceability and Comparability Relationships for Results for 5 Analytes in 5 Laboratory Measurement Systems during 4 Months

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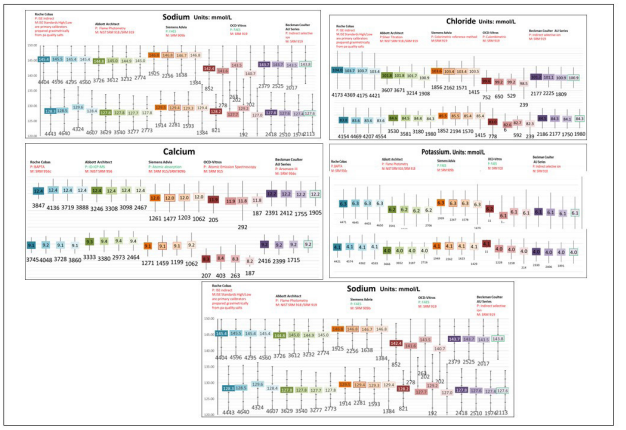
Background: The metrological traceability of the test in medical laboratories is key to contribute to comparable and reliable results, but still the awareness of this feature is not demanded in the majority of the laboratories. The JCTLM publish continually the Reference materials and Reference procedures approved for an important number of tests **Methods:** The results obtained from Bio-Rad Unity Worldwide Report during 4 months (February to May, 2019) in 5 measurements systems, Cobas-Roche, Vitros-Ortho-Clinical Diagnostics, Advia-Siemens Healthineers, Abbott Architect and Beckman Coulter with "Bio-Rad Lyphochek Assayed Chemistry Control" lot 26430 in 2 levels and their relationship with the metrological traceability for material and procedure for 5 analytes was analyzed comparing means, Standard Deviations and plotted on a box plots. The information for materials and procedures used by each manufacturer was compared with the JCTLM database. **Results:** Magnesium was traceable to SRM 956 and SRM 929 materials, Chloride was traceable to SRM 919, Calcium was traceable to SRM 956c, SRM 956 and SRM 915, Potassium was traceable to SRM 918, SRM 909b, Sodium was traceable to SRM 919, SRM 909b, but anyone of the 5 systems were traceable to the materials endorsed by the JCTLM. **Conclusion:** There are reference materials commonly and although they are not always traceable to those endorsed by the JCTLM, the results are harmonized in several cases. The use of Bio-Rad's third opinion quality material allows visualizing the harmonization of the results among various measurement systems, because can be used cross-sectionally in various measurement systems, becomes a valuable tool to assess harmonization between the results obtained in different measurement systems. The Bio-Rad UNITY interlaboratory comparison report is a valuable tool in the evaluation of the harmonization of the results obtained by different measurement systems.



B-258

Use of Lean Principles to Optimize Critical Value Reporting and Notification

J. Sekol, M. Wiseheart, J. Biksacky, J. Copeland, J. Carey, B. Cook, M. Tuthill, G. Winston-McPherson, L. Dwyer, D. Smith, J. Naderi, J. Zajechowski, Z. Abouarabi, K. Burzynski. *Henry Ford Health System, Detroit, MI*

Background: Our large Midwestern tertiary care institution reports approximately 150 critical values (CV) each day, or nearly 55,000 per year. Results for the 35 tests that have defined CV are released into the electronic medical record (EMR) by the testing technologists after performing the system required verbal notification step with the responsible provider. Our established goal for reporting CV was 98% within 60 minutes, but in actuality 98% were reported in 27 minutes. Although we were meeting the goal, we examined whether the delay for release of result and notification to the provider could be decreased to allow faster decision making and potentially improve patient care. Our new goals were to immediately release the CV into the EMR and to decrease our notification time to <20 minutes. **Methods:** The project team analyzed over 1,000 CV from a variety of inpatient and outpatient reports. Using Lean Management principles and tools such as the plan/do/check/act (PDCA) process improvement cycle allowed us to discover potential gaps and use root-cause analysis to identify the contributing factors for the 27 minute delay. A module associated with our laboratory information system (LIS) was employed to facilitate critical value reporting. **Results:** Three workflow issues were identified that could be modified to decrease the delay; 1) Change our protocol to release CV into the EMR immediately upon recognition, with verbal notification comments to follow, 2) Standardize the documentation process to improve understanding of entries into the patients' charts, 3) Reassign the CV notification and documentation process to the Laboratory Customer Service (LCS) Department, where the responsibilities of continuous flow of patient testing are removed. This required an electronic means to alert the team as to which patient results required action. To support the workload and expand hours of service to 24/7, LCS obtained and trained 6 additional employees. By removing the CV process from the Core Laboratory, the potential number of people involved in the notification procedure decreased by 81%, facilitating better control of documentation and standardization processes. CV are released sooner into the EMR and providers are notified nearly instantaneously of their patient's potentially life-threatening laboratory value. CV from one month after the new process was implemented (n=3500) demonstrated a median release of result-to-notification time of 5 minutes. 90% of CV were communicated and documented within 9 minutes and 98% were completed in 17 minutes. **Conclusions:** We virtually eliminated the time from release of result to posting in EMR which will now

enable more rapid decision making by providers. Our multidisciplinary team used Lean tools to decrease the time between result and notification by 10 minutes for each CV, or 1,500 minutes each day.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Toxicology & Therapeutic Drug Monitoring

B-261

Investigation of Plasma as an Alternative Matrix for the Detection of Lorazepam using the Roche Urine Screening Assay for Benzodiazepines

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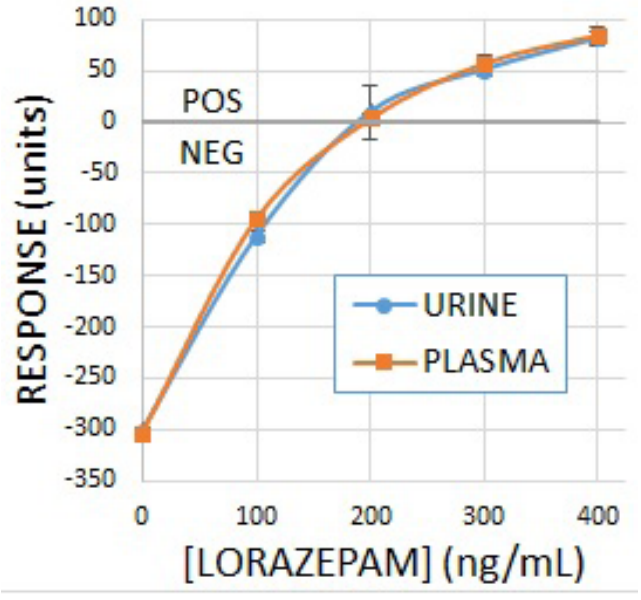
Background: We received an inquiry regarding a screen-negative for urine benzodiazepines (Roche Cobas Benzodiazepines Plus KIMS immunoassay) in an inpatient receiving regular prescribed doses of lorazepam (Ativan), 1 mg q6h. It is well known that qualitative urine immunoassays for benzodiazepines are often insensitive for detection of lorazepam, including very low cross-reactivity of the glucuronide metabolite. For numerous reasons, plasma as an alternative matrix on a urine KIMS immunoassay might have different analytical sensitivity for a given drug. We investigated this possibility for detection of plasma lorazepam using the Roche urine benzodiazepines screening assay. **Methods**: Matrices used for spiking with lorazepram were: (A) pooled urine, known to be drug-free, and (B) pooled plasma, which was indistinguishable from A as assessed for lorazepam by LC-MS/MS. A and B samples were spiked to identical lorazepam concentrations (Cerilliant; l0-400 ng/mL) and measured using the Roche urine benzodiazepines screening assay. Numerical response curves vs. lorazepam concentration were compared between A and B (n=4). **Results**: Numerical response of the assay is calibrated to the response to 100 ng/mL nordiazepam = 0 units. Numerical response curves for A and B were essentially indistinguishable **(Figure)**. Qualitative positivity for the assay corresponded to approximately 200 ng/mL lorazepam, in moderate agreement with a kit insert value of 163 ng/mL. Measurement of B would have been insufficient for detection of lorazepam in this patient, as plasma concentrations by LC-MS/MS over the course of 12 days never exceeded 50 ng/mL. These data were consistent with existing literature on usual prescription-based plasma concentrations of lorazepam. **Conclusions**: Experiments to compare the numerical response of the Roche urine benzodiazepines screening assay between lorazepam-spiked plasma and urine samples demonstrated that the assay was equally sensitive to lorazepam in either matrix. Assay sensitivity was too low, however, to detect usual concentrations of lorazepam in patient plasma.



B-263

A Semi-Targeted Approach for Detecting Fentanyl Analogs in Urine Using Precursor Ion Scan Mode

S. M. Roper, M. M. Budelier. *Washington University School of Medicine, St. Louis,, MO*

Background: The opioid crisis has prompted the interest of many providers in the capabilities of urine drug testing (UDT) to detect fentanyl analogs (FA). However, the most frequently used methods for clinical UDT are not designed to broadly screen for the variety of FAs that may be encountered. Using the knowledge that fentanyl and many FA fragment to common product ions of m/z=105 and m/z=188, we developed and tested a semi-targeted UDT for FA using precursor ion scan (PIS) mode. **Methods:** 94 residual adult urine specimens were screened for the presence of fentanyl and 6 FA using PIS. A Waters Xevo TQD tandem mass spectrometer was configured to acquire data in positive mode on all precursor ions 200-400 Da triggered by the detection of product ions m/z=188 and m/z=105. Data was post-processed to evaluate extracted ion chromatograms that correspond to known FA masses, including precursor ions of m/z=281 (4-ANPP), m/z = 323 (Acetylfentanyl), m/z=335 (Acrylfentanyl), m/z= 337 (Fentanyl), m/z=351 (Butyrylfentanyl), m/z=369 (4-FIBF), and m/z= 375 (Furanylfentanyl). Presumptive positivity was assigned based on visual assessment of chromatograms and estimated retention time windows for each FA. Performance of the PIS method was assessed by comparison to a targeted LC-MS/MS method for fentanyl and acetylfentanyl (addition of other FAs to the targeted profile is in progress). **Results:** Relative to the targeted assay, the sensitivity of the PIS screen was 91% and 100% for fentanyl and acetylfentanyl, respectively. The specificity of the PIS method for fentanyl was 97% while acetylfentanyl specificity was 95%. The accuracy of PIS was 94% for fentanyl and 96% for acetylfentanyl. Although not yet verified, 1 presumptive positive for acrylfentanyl and 7 specimens containing 4-ANPP were also identified by PIS. Interestingly, all specimens tentatively positive for FA were concurrently positive for fentanyl. In addition, all presumptive 4-ANPP identifications were observed in samples confirmed positive for acetylfentanyl. On-going studies include assessment of PIS relative to targeted LC-MS/MS, method optimization to decrease detection limits/reduce isobaric interferences, and expanding data review to screen for additional FA. **Conclusion:** Preliminary results suggest that semi-targeted screening for FA by PIS is a viable option for discovering previously undetected compounds in adult urine, however studies to assess the performance characteristics of this method are on-going. This novel approach to UDT has potential implications for public health surveillance and improved patient care in toxicological evaluations. We envision application of the PIS screen as a tool to identify FA present in our community which could be added to our targeted LC-MS/MS UDT.

B-264

Determining the Effective Compounds of Salvia Miltiorrhiza through HPLC Fingerprinting Coupled with Chemometrics

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Background: *Salvia miltiorrhiza* Bunge (Danshen) is a well-known herb in traditional Chinese medicine. Scholars have sought to identify the major active components of the plant that give it various biological properties, such as antioxidant and antitumor activities. This study assessed the chemical composition and biological activities of *S. miltiorrhiza*. To investigate the spectrum-effect relationship between the high-performance liquid chromatography (HPLC) fingerprints and bioactivities of *S. miltiorrhiza*.

Methods: HPLC fingerprints of 23 *S. miltiorrhiza* lines were established and analyzed using hierarchical clustering analysis. The antioxidant activity of *S. miltiorrhiza* was evaluated using the potassium ferricyanide [$(K_3Fe(CN)_6$] reduction method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. The antitumor activity was measured using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay in human alveolar lung epithelial carcinoma (A549) and human ovarian clear cell carcinoma (TOV-21G) cancer cells. Results of the antioxidant and antitumor activities were used for principal component analysis (PCA). Multiple linear regression (MLR) analysis was performed to establish HPLC fingerprint-bioactivity relationship to explore the components of *S. miltiorrhiza* extracts that contribute considerably to antioxidant and antitumor activities.

Results: HPLC fingerprints as well as PCA analysis using four different bioactivities illustrated that the 23 *S. miltiorrhiza* lines can be categorized into four groups. An MLR analysis indicated a close correlation between the fingerprints and antioxi-

dant activities. Compounds B, D (salvianolic acid B), G (cryptotanshinone), H, K, and N had considerably positive effects on the antioxidant activity of iron chelators $K_3[Fe(CN)_6]$. Compounds B, E, and G showed a positive impact, whereas compound M showed a negative impact on DPPH radical-scavenging activity. Moreover, compounds J (dihydrotanshinone), K, and P (tanshinone IIA) had considerably positive effects on the cytotoxicity of A549 cancer cells; compounds B, C, and P (tanshinone IIA) had considerably positive effects on the cytotoxicity of TOV-21G cancer cells. Adjusted-R-squared parameters for the four MLR models were 0.696, 0.652, 0.741, and 0.758, respectively.

Conclusion: This work provides a general model for the exploration of spectrum-effect relationships by assessing HPLC fingerprints of *S. miltiorrhiza* in combination with its antioxidant and antitumor activities. This strategy seems to be the most promising for assessing a plant material.

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New Second Generation Specimen Validity Testing for Urine Drugs-of-Abuse Testing

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BackgroundDuring the past decade, the percentage of positive urine drugs-of-abuse (DOA) tests has progressively declined while the overdose death rate and the number of opioid doses have steadily increased. The subversion of urine DOA screening is a major problem. Due to ineffective pre-analytical subversion detection, the first generation of specimen validity tests (SVTs) can only detect 2 of 18 classes of subversion.

ObjectivesA principal objective of the VDx GEN2-SVT™ panel is to increase the effectiveness of pre-analytical SVT testing to include 18 classes of subversion. Secondarily, the detection of subversion should be viewed as presumptive evidence of SUD. These objectives can be achieved without undue burden on laboratories by adding only 2 new tests to the current panel of SVTs commonly used on automated instruments.

MethodsThe sequence of testing for pre-analytical assessment of urine specimens for subversion would be: **1.** Perform a VDx GEN2-SVT™ Urine Creatinine screen. This screen contains a decolorizing reagent which minimizes interferences from hemoglobin. This screen, in conjunction with #2 below, can be used to help differentiate dilution, in vivo creatine/protein water loading, salting or acid adulteration.**2.** Perform a VDx GEN2-SVT™ Specific Gravity Index Screen. Interpretation of this result should be used in conjunction with #1 above.**3.** Perform VDx GEN2-SVT™ True Urine LD, True Urine SD and Oxidant History screens. These screens help to differentiate dilution, oxidation, use of synthetic urine or substituted urine.**4.** Perform VDx GEN2-SVT™ pH screen. This screening reagent contains dual indicator dyes that provide more accurate readings near pH 3.0 and pH 10.5.

ResultsThe analysis of ~ 5,000 urine specimen results from several clinical laboratories using the VDx panel showed that ~ 10 - 20% of urine specimens had indicators of subversion and were considered "invalid" for subsequent urine DOA screens. This is in contrast to ~ 5% urine specimens showing subversion by traditional methods. Among the invalid urine specimens, many were determined to be substituted or synthetic urine by both the True Urine LD and SD tests. Some of these urine specimens screened positive for the prescribed drug class. However, LC/MS-MS analysis confirmed the presence of the parent drug but no metabolites of the parent drug which is consistent with drug spiking.

ConclusionsThe GEN2-SVT™ panel increases the effectiveness of pre-analytical SVTs to include 18 classes of subversion commonly used by individuals with a substance use disorder. The increased effectiveness can be achieved without undue burden on laboratories by adding only 2 new tests to the current panel of tests. Urine specimens passing the pre-analytical validity tests can undergo urine DOA screening with confidence. Urine specimens from donors failing subversion testing by the VDx GEN2-SVT™ panel should be considered for testing by LC/MS-MS to broaden the number of drugs detected.

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Six-Year Trends in Drug Use: Urine Drug Screening Positivity Rates for Community-Based Patients in Ontario, Canada from 2014 to 2019

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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:** LC-MS/MS urine drug screening results from N>612,000 tests performed between January 1, 2014 to December 31, 2019 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 six-year testing period, urine drug screening positivity rates ranged from 76.6% (cotinine) to <0.01% (7-aminoflunitrazepam, benzylpiperazine, desalkylflurazepam, diazepam, flunitrazepam, MDEA, MDPV, mephedrone, phenazepam and triazolam). From 2016 to 2019, annual significant ($p \leq 0.05$) positivity rate decreases were observed for levamisole (8.0% to 2.5%). Cocaine consumed by this patient population has annually contained significantly less levamisole since 2016. Cocaine use itself has also decreased since 2017, while fentanyl use has increased. From 2017 to 2019, significant positivity rate changes were observed for: benzoylecgonine (13.3% to 11.4%); buprenorphine (9.7% to 12.1%); cotinine (72.3% to 66.8%); hydromorphone (10.0% to 7.0%); lorazepam (5.0% to 4.3%); naltrexone (0.3% to 1.1%); norcodeine (5.9% to 5.4%); norfentanyl (6.8% to 8.3%); noroxycodone (15.9% to 13.2%); oxazepam (6.0% to 4.7%); and oxycodone (13.5% to 10.5%). Lorazepam, oxycodone and hydromorphone use dropped significantly from 2017 to 2019. Annual decreases in nicotine use were also observed. Naltrexone use has increased over the same three-year period. From 2018 to 2019, significant positivity rate changes were observed for: diphenhydramine (12.0% to 10.5%); and temazepam (4.6% to 4.0%). Relative to the 2019 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. Suboxone therapy has increased recently but evidence of methadone-based opioid antagonist therapy remained more common in 2019. In 2019, methadone, EDDP, buprenorphine, norbuprenorphine and naloxone positivity rates were 31.3%, 31.6%, 12.1%, 13.4% and 12.2% respectively. **Conclusions:** This retrospective review of qualitative LC-MS/MS urine drug screening positivity rates from 2014 to 2019 identified several significant annual drug use changes. Laboratories can provide detailed information on licit and illicit drug use trends within a specific patient population by tabulating, interpreting and communicating urine drug screening positivity rates to their clinical communities.

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Exploration of Applying High-Resolution MS^2/MS^3 Mass Spectrometry for Screening Toxic Natural Products

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Background: Many natural products have biological effects. Poisoning caused by natural products is often found in clinical toxicology cases. Until recently, the diagnosis of natural product poisoning was primarily presumptive, and many cases have likely gone undiagnosed. In order to identify natural toxins in clinical cases, a screening method with structural resolving power is in demand, given the complex molecular structures of many natural products. We established a liquid chromatography-multi-stage-mass spectrometry ($LC\text{-}MS^n$) assay with hierarchical MS^2/MS^3 mass spectrum acquisition as a screening method for natural toxins. Besides using the accurate mass and isotope pattern, multiple rounds of fragmentation in a collision cell was carried out to generate multi-level fragments of a compound. The delicate structural information obtained in MS^2/MS^3 mass spectra can play a key role in compound identification by matching the fragmentation patterns to a spectral library. Thus, the compound identification power of mass spectrometry can be enhanced in resolving the clinical cases.

Methods: The $LC\text{-}MS^n$ assay was carried out using an Orbitrap ID-X instrument (Thermo Fisher Scientific, San Jose, CA). The separation was achieved by using an Accucore C18 column (3.1 mm x 100 mm, 2.6 µm particle) with gradient elution (MPA: 5 mM ammonium formate in water with 0.05% formic acid; MPB: MeOH : ACN 1:1 with 0.05% formic acid). The ion source was ESI in positive ion mode. Data-dependent acquisition (DDA) mode was employed, with each cycle of full scan in mass range m/z 10-1000, 10 precursor ions selected for MS^2 scan, and 3 precursor ions selected for MS^3 scan. A spectral library with MS^2/MS^3 mass spectra was

constructed. It consisted of 124 natural products encompassing central nerve system (CNS) stimulants, CNS depressants, neurotoxins, cardiotoxins, nephrotoxins, hepatotoxins, gastrointestinal toxins, and hematologic toxins from those plants often encountered in clinical cases. Serum samples were mixed with ACN to precipitate proteins, and the supernatants were dried and reconstituted in the sample diluent for LC-MS^n analysis. Urine samples were diluted in the sample diluent for LC-MS^n analysis.

Results: The limit of detection (LOD) of the LC-MS^n assay was verified in both serum and urine matrices. For each compound, the LOD was evaluated from 1.0 ng/ml to 1000 ng/ml for urine samples and from 0.50 ng/ml to 500 ng/ml for serum samples. The LODs demonstrated good analytical sensitivity of the LC-MS^n assay and the accuracy of compound identification at low concentrations. The performance of the LC-MS^n assay was compared with a previously established conventional LC-MS^2-only assay, and certain advantages were observed. The LC-MS^n assay was successfully applied to identify the culprits in clinical cases, and the utility was shown in real examples.

Conclusion: The LC-MS^n assay demonstrated the value of applying high-resolution MS^2/MS^3 mass spectrometry for screening toxic natural products. It can be an ideal complement to conventional LC-MS^2-only assays. In particular, the use of MS^3 mass spectra in spectral pattern matching helped resolve structural isomers that are widely present in natural products. In addition, an exclusion list of endogenous compounds was tested to rule out interferences in sample matrices, and promising results were obtained.

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Evaluation of Clinical Performance of ARK™ Methotrexate Immunoassay on Roche cobas 8000 c502 Analyzer --- Platform Switch Experiences of a Medical Center in Taiwan

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IntroductionMethotrexate (MTX) is an antifolate drug commonly used for the treatment of malignant disease, such as acute lymphoblastic leukemia, non-Hodgkin lymphoma and osteosarcoma. To avoid the toxicity of high-dose treatment, administration of MTX is followed by leucovorin rescue therapy. In clinical practice, therapeutic monitoring of serum MTX concentration is crucial for toxicity assessment. As the clinical decision point for patient discharge is based on a MTX concentration <0.05 µmol/L, assays with excellent precision and accuracy in the low measuring ranges are necessary. Our laboratory has performed MTX assay on Abbott system for more than 15 years. In October 2019, we implemented the Roche total laboratory automation system, which was a challenge for us to switch the MTX assay platform due to the lower sensitivity of ARK™ MTX assay (0.04 µmol/L) compared to that of Abbott MTX assay (0.02 µmol/L). In this study, we evaluated the clinical performance of ARK™ MTX assay on a Roche c502 Analyzer, especially in the ranges of clinical decision-making concentration. **Methods**Assay validation studies including accuracy, precision (between-day/within-run), analytical measurement range (AMR), sensitivity (LoQ), and method comparison were performed according to the accreditation requirements of the College of American Pathologists (CAP). Quality control materials with high, medium, and low levels were respectively measured at least 20 times in three consecutive days. The assay mean, standard deviation (SD), coefficient of variation (CV) and bias were analyzed. Serum specimens obtained from sixty patients with MTX concentration ranging from 0.02 to 1,000 µmol/L were analyzed with Architect MTX and ARK™ MTX assays for comparison and calculate between-day CV at concentration around 0.05 umol/L.with 10 replicates.**Results**Our results showed that the precision of ARK™ MTX assay was acceptable, of which the within-run precision at low, medium and high levels were 6.11%, 1.62%, and 3.02%, and between-day precision

were 6.71%, 1.44%, and 2.23%, respectively. The between-day CV at clinical decision-making concentration (0.05 µmol/L) was 16.1%, fulfilling the criterion of less than 20%. The accuracy evaluation also showed acceptable results with the bias 10% at 0.07 µmol/L, 6.25% at 0.4 µmol/L, and -1.06% at 0.8 µmol/L. For AMR verification, patient samples were used to prepare with different levels of MTX ranging from 0.04 µmol/L to 1.25 µmol/L. The results were acceptable with bias < 15% and CV < 10% at each level. For assay comparison, the results showed a good correlation between Architect MTX and ARK™ MTX assays with $r^2 = 0.999$.

ConclusionOur data demonstrated that the performance of ARK™ MTX assay operating on Roche TLA system is suitable for clinical service. We have successfully provided ARK™ MTX assay on a 24 hours-a day / 7 days-a week basis, which is sufficient to meet the clinical demands of a medical center in Taiwan.

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Accordance of Capillary and Venous Blood Sampling for Therapeutic Drug Monitoring and the Basic Metabolic Profile

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Background: Current practice utilizes venous blood drawn from a peripheral vein to obtain both a basic metabolic profile and a vancomycin drug concentration. Timing of the vancomycin must correlate with the dosing schedule for accurate therapeutic drug monitoring (TDM); however, this may not always align with the routine blood draws required for patient care. Additional sampling routinely occurs to ensure accurate results. These excessive blood draws may cause unwarranted patient discomfort, hospital-acquired anemia, and increased hospital cost. Our goal in this study was to determine the comparability of capillary vs. venous blood draws in measuring serum vancomycin concentrations and the basic metabolic profiles (BMP). **Methods:** In this prospective observational study, we measured paired vancomycin and BMP venous and capillary plasma concentrations in patients who were undergoing vancomycin therapy and provided informed consent. Capillary sample collection was coordinated to occur just after or prior to (+/- 15 mins) their venous blood draw for vancomycin TDM. Venous blood was collected in Lithium Heparin blood collection tubes (Vacutainer; BD). Capillary puncture was done with Microtainer high flow lancets and a minimum 0.2-mL blood was collected in 0.6-mL Lithium Heparin tubes (Microtainer; BD). Vancomycin was measured by a competitive kinetic interaction of microparticles in a solution (KIMS) assay on a Roche Cobas C501 analyzer (Roche). Assays were performed according to the manufacturer's instructions. To determine the variation between the two sample types, we performed Deming regression and Bland-Altman analysis. **Results:** Fifty-nine samples from 57 unique paired patients' plasma concentrations were collected. Analysis for fifty-nine paired capillary vancomycin (y) and venous (x) samples by Deming regression yielded the equation: $y = 0.92x + 1.00$ µg/mL. The 95% confidence intervals were −0.23 to 2.23 µg/mL for the intercept and 0.83 to 1.00 for the slope. Differences between paired specimens were determined not to be significant; P=0.41. The mean difference between the paired concentrations were 0.23 µg/mL (95% confidence interval -0.78 to 0.32) and there was no evidence of concentration dependent bias. Similarly, seven of eight basic metabolic profile analytes also showed no statistically significant differences between venous or capillary sampling. However, there was a significant difference in potassium concentrations with a mean bias of 1.6 mg/dL higher values observed in capillary vs. venous samples; P<0.0001. This large difference is likely attributable to a high degree of hemolysis observed during fingerstick capillary sampling and was observed throughout the duration of the study. **Conclusion:** Capillary and venous samples for vancomycin and seven of eight analytes in the BMP were highly comparable amongst the patients we tested. Capillary sample collection represents a viable option for testing these analytes and consideration should therefore be given to allowing this specimen type in the rare situation where venous access is not possible.

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Differentiation of Marijuana Users from Individuals Taking Dronabinol by Evaluating Urinary Cannabinoid Profiles using LC-MS/MS

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Introduction: The therapeutic potential for delta-9-tetrahydrocannabinol (THC), the active ingredient in dronabinol, has been the topic of several recent pain management studies. As with other pain therapies, monitoring the metabolic profile of the patient is critical to determine compliance. This is particularly challenging with dronabinol because THC is also present in marijuana. Therefore, monitoring the minor cannabinoids of marijuana, which are not present in dronabinol, has been proposed. In this study, we developed a rapid LC-MS/MS assay with minimal specimen preparation to quantitate 11 major and minor cannabinoids in urine. Using this assay we determined the urinary cannabinoid profiles of dronabinol, marijuana, and negative controls. **Methods:** Specimens from patients enrolled in a clinical trial using dronabinol (n=4) were obtained prospectively. Patients were required to abstain from marijuana use during the study and were only administered dronabinol after a 2 week washout period in which they screened negative for THC metabolites by immunoassay. Residual clinical urine specimens were used for the marijuana (n=55) and negative control groups (n=31), which were positve and negative by THC ELISA, respectively. Samples were digested with B-glucuronidase for 5 minutes, diluted 1:1.5 in methanol and injected without extraction. Metabolites were separated by UPLC with a 5 minute gradient elution.

A Waters Xevo TQ-S triple quadrupole mass spectrometer operated in negative ion mode was used to quantitate major (THC-OH, THC-COOH) and minor cannabinoids (TCHV, THCA-A, CBN, CBC, CBD, CBDA, CBDV, CBG, CBGA). **Results:** THC-COOH was detected by LC-MS/MS in all marijuana user samples and at concentrations below the immunoassay cutoff in two samples from the non-cannabinoid group. Of positive THC-COOH samples, at least one minor cannabinoid was detected in 91% (52/57) with several detected in most specimens. The presence of minor cannabinoids was variable but in general were detected more frequently in samples with higher THC-COOH concentrations. CBG (79%, 45/57) and CBD (74%,42/57) were the most consistent minor cannabinoids detected while the rate of detection in the remaining minor cannabinoids ranged from 2-25%. CBDA was not detected in any samples. In the dronabinol group, no cannabinoids were detected prior to dronabinol dosing. During the dronabinol dosing period, all four subjects had quantifiable concentrations of THC-OH and THC-COOH while no other cannabinoids were detected. **Conclusions:** Minor cannabinoids were not detected in any dronabinol urine specimens but were detected in nearly all of the marijuana group specimens indicating a panel of minor cannabinoids can be used to evaluate compliance with dronabinol therapy with reasonable confidence. Although the sample size of this study was limited and the source of the non-dronabinol THC (i.e. the marijuana group) was not known, the consistent presence of CBG and CBD in these specimens suggests thier utility as indicators of non-compliance in the context of dronabinol therapy.

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Development of a Fentanyl Analogue Screening Library for High-Resolution Mass Spectrometry

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INTRODUCTION: The overdose of illicitly manufactured synthetic opioids including fentanyl and analogs has escalated significantly in the US in recent years. In clinical practice, detection of synthetic fentanyl analogs could be undetectable by either immunoassay or LC-MS/MS methods with a limited library. In some cases, symptoms are consistent with an opioid overdose and resolve with naloxone treatment, however no drug culprits are identified with routine testing. To address these issues, the Centers for Disease Control and Prevention developed Traceable Opioid Material Kits to support laboratory detection of current and emerging opioids. **OBJECTIVES**: The objective of this project was to use the CDC FAS kit to develop a high resolution mass spectral FAS library and validate our routine comprehensive drug testing method for limit of detection (LOD) and matrix effects (ME) in urine and serum for the 150 synthetic fentanyls/opioids. The resulting library and validated method were evaluated for their ability to identify synthetic opioids in routine clinical. **METHODS**: The FAS kit (*Cayman Chemical)* includes 120 synthetic fentanyl compounds and 30 synthetic opioid compounds. Chromatographic separation was performed using a Kinetex C18-column with a 10-minute gradient from 2%-100% organic; and data was collected on a SCIEX TripleTOF®5600 operating in positive-ion mode using a TOF-MS survey scan with IDA-triggered collection of product ion spectra. Urine samples were prepared by a 1:5 dilution in mobile phase and serum samples (250 µL) by protein precipitation with acetonitrile. LOD was evaluated by duplicated injections of compound standards at different concentrations in drug-free urine and serum samples. LOD was defined as the lowest concentration for a compound identified with a combined score >70% and signal-to-noise ratio >20:1, in duplicate injections. ME were determined by spiking drug standards into drug-free urine (10 ng/mL) or pooled drug-free serum (2.5 ng/mL) of six healthy subjects in triplicates, and comparing to drug standard spiked into water. **RESULTS**: A mass spectrum was acquired and added to our in-house HRMS library for all analytes in the FAS kit. Further analysis of the acquired spectrum revealed characteristic fragmentation patterns based on structure that can be used for structure elucidation and compound identification by laboratories that do not have access to the FAS kit. The LODs ranged from 0.5-10 ng/mL (median, 2.5 ng/mL) in urine and 0.25-2.5 ng/mL (median, 0.5 ng/mL) in serum. ME were significant for a select few analytes which is to be expected given the simple sample preparation techniques used (dilution or protein precipitation). They ranged from -79%–86% (median, -37%) for urine and -80%–400% (median, 0%) for serum. The library has been used to retrospectively analyze data files from past clinical toxicology cases, drug products evaluated in the laboratory, and urine samples that screened positive by a fentanyl immunoassay in the emergency department. Select cases will be reviewed in the presentation.**DISCUSSION:** The FAS library can now be used to retrospectively and prospectively analyze cases of suspected fentanyl exposure to aid in furthering our understanding of the ongoing epidemic.

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First Successful Comparison of Quantum Blue® Rapid TDM Assay Standardization with WHO International Standard for Infliximab

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Background: Therapeutic drug monitoring of RA or IBD patients under anti-TNF therapy is based on trough level determination of the drug. Rapid assays and multiple ELISAs are available that measure anti-TNF biologics. An international standard is required to improve comparability among different assays. Recently, WHO introduced a series of anti-TNF standards for etanercept, adalimumab and infliximab. This is the first step for achieving common standardization of assays available on the market. The aim of the study was to evaluate the correlation of the WHO standard with BÜHLMANN Quantum Blue® Infliximab standardization and to compare spiking recovery in three commercially available infliximab ELISAs and the rapid test Quantum Blue® Infliximab.

Methods: Calibration curves were generated with BÜHLMANN calibrators and with calibrators made from WHO international standard for infliximab (NIBSC 16/170). Twenty-six serum samples, covering a concentration range from 0.5 µg/mL to 19 µg/mL, were analyzed with both calibration curves and compared by Bland-Altman and Passing-Bablok analysis. Furthermore, recovery of six serum samples spiked with WHO international standard for infliximab was determined in Theradiag LISA TRACKER Infliximab (a), Grifols/Progenika Promonitor-IFX (b), Immundiagnostik IDKmonitor Infliximab drug level (c) and BÜHLMANN Quantum Blue® Infliximab (d). Spiking recovery experiments were performed according to Westgard 2008.

Results: The sample values gained with BÜHLMANN calibrators showed an excellent correlation with values gained with the WHO international standard for infliximab as calibrator. Passing-Bablok regression analysis revealed a slope of 0.96 and correlation coefficient (R) of 0.99. Bland-Altman analysis revealed a mean difference in the obtained values of less than five percent. Regarding spiking recovery analysis, all tests exhibit an excellent mean recovery of 101% (85-114%; a), 99% (91-105%; b); 101% (95-107%; c) and 94% (88-100%, d).

Conclusion: Current standardization of Quantum Blue® Infliximab rapid test correlates very well with the WHO international standard for infliximab (NIBSC 16/170). Spiking recovery was highly comparable for ELISAs and the Quantum Blue® Infliximab assay but the rapid test brings the advantage of fast time-to-result and simplicity of usage in a more patient near medical environment.

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A Validated UPLC-MS/MS Method for Therapeutic Drug Monitoring of Mycophenolic Acid in Renal Transplant Patients

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Background: Mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS) are specific anti-lymphocyte proliferative drugs. The use of MMF or EC-MPS as an adjuvant therapy with the calcineurin inhibitor agents after kidney transplantation is very common. MMF and EC-MPS are hydrolyzed to mycophenolic acid (MPA) after administration. A given dose of MMF or EC-MPS can result in different plasma concentrations which may lead to sub-therapeutic drug exposure or increase adverse drug reactions at excessive plasma concentrations. Our study aimed to develop a method to measure MPA and its metabolites (mycophenolic acid glucuronide, MPAG) by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for patients after kidney transplantation.

Methods: The calibration curve samples were prepared by spiking drug free plasma with MPA and MPAG. A 10 µL aliquot of calibrators or samples was transferred into a centrifugation tube. After addition of the stable isotope labeled internal standards (MPA-d3 and MPAG-d3) and protein precipitation, the supernatant was 10-fold diluted and injected into a chromatography system consisting of Xbridge Direct Connect HP C18 (2.1x30 mm, 10 µm) and ACQUITY UPLC BEH C18 (2.1x50 mm, 1.7 µm) with gradient made of mobile phase (5 mM ammonium formate pH3.5 in water and 100% methanol). 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 linearity of MPA and MPAG were between 0.3-13.6 µg/mL and 2.6-232.9 µg/mL (r2 > 0.999). The imprecision for within-run and between-run was less

than 5.8%. The accuracy was evaluated by spike recovery and the mean recovery was 88%-109%. No carryover and ion suppressant or enhancement was observed in this validated method. The chromatography run time was 6 min. The developed on-line SPE coupled with UPLC-MS/MS method was used to quantify 351 plasma samples from renal transplant patients after MMF or EC-MPS administration. The MPA and MPAG concentrations vary markedly individual patients after equal dose. These results indicated that monitoring of MPA and its metabolite in plasma level is required. By comparing 351 plasma samples, we also found that immunoassay overestimated the concentration of MPA, averaging 15.1%.

Conclusion: A fast and accurate on-line solid phase extraction coupled with UPLC-MS/MS method was developed and successfully applied for routine therapeutic drug monitoring purposes for patients treated with MMF and EC-MPS.

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Selective, Simple and Fast Determination of Everolimus in Whole Blood for Therapeutic Drug Monitoring

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Background: Everolimus is an immunosuppressive drug used in transplants recipients. This kind of drugs is successfully applied in many organ transplantation to avoid the organ rejection. It has a narrow therapeutic index and show the desired therapy effect with acceptable tolerability only within a narrow range in blood concentrations. At low blood levels, there is a risk of organ rejection. At high blood levels, serious side effects may arise, including nephrotoxicity, cardiotoxicity, neurological effects and elevated risk infections. The correlation between drug concentrations and clinical outcomes is an important factor for the use of therapeutic monitoring of immunosuppressive medications. Several studies has been demonstrated the benefit of this monitoring. Clinical trial methods that guarantee speed and selectivity are increasingly necessary in monitoring this drug.The aim of this study was to develop and validate a simple, rapid and sensitive LC-MS/MS method for quantification the Everolimus in whole blood. **Methods:** In this method, 100 µL of whole blood was precipitated with a solution of zinc sulfate and acetonitrile. Detection was performed on a Waters XEVO TQD mass spectrometer with electrospray ionization (ESI+) by monitoring the fragmentation ions of 975.8→908.6 (m/z) and 975.8→926.6 (m/z) for Everolimus and 979.5→912.5 (m/z) for Everolimus-d4. Chromatographic separation was performed on a Waters ACQUITY UPLC equipped with Ascentis Express C18 column and using a mobile phase constituted by methanol with 0.1% of formic acid and 5 mM of ammonium formate at a flow rate of 250 µL/min. **Results:** Everolimus was determined with a retention time of approximately 0.94 minutes. The linear analytical range achieved was between 2.0 and 40.0 ng/mL. The medium range of recovery obtained was between 98 and 108% and the inter and intra-day imprecision were less than 5.1 % for Everolimus. **Conclusion:** The LC-MS/MS method developed was a simple, rapid and sensitive to detect Everolimus in a whole blood. The method was successfully validated and can be applied to therapeutic drug monitoring of this immunosuppressive drug.

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Rapid Determination of Methotrexate in Human Blood by LC-MS/MS

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Background: Methotrexate (MTX) is widely used as an antiproliferative agent in treatment against cancer and as an anti-inflammatory mainly for rheumatoid arthritis. Its pharmacokinetics is characterized by great inter-individual variability. This factor influences the clinical results as well as the risk of toxicity. Mass spectrometry (MS) is a highly specific and sensitive technique used to detect many different analytes with several chemical characteristics, including MTX. This technique has been widely used by clinical laboratories for quantification of small molecules and, by extension, was used for therapeutic monitoring of medicines. It is an attractive alternative to immunology methods, as it is not subject to the same interferences. A joint LC-MS / MS method can be even better at for the detection and quantification of methotrexate in human blood, as described later. The aims is to Develop and validate a LC-MS/MS method for determination of methotrexate levels in human blood. **Methods:** Chromatographic separation was performed on a Waters ACQUITY UPLC equipped, with a Ascentis Express C18 column and using a mobile phase constituted by methanol with 0.1% of formic acid and 5 mM of ammonium formate at a flow rate of 250 µL/min. Analysis was made on Waters XEVO TQD mass spectrometer with electrospray ionization (ESI+) for quantification of Methotrexate. The extraction procedure is an addition of internal standard solution (Methotrexate-d3) and then a simple protein precipitation with organic solution. In total, 27 real samples were analyzed by immunoassay (Architect©, Abbot) and in the LC-MS/MS method, and the results were compared by Passing-Bablok regression and Bland-Altman analysis. **Results:** The total chromatographic run time obtained was 1.0 min. The linear range achieved was between 0.05 and 100.0 µmol L^{-1}. The medium range of recovery was between 95 and 102%. The coefficient of variation ranged from 1.5 to 3.8% and the limit of detection (LOD) calculated was 0.05 µmol L^{-1}. The linear regression equation was LC-MS/MS = 0.969489 *(Imunoassay) + 0.000945843. The correlation coefficient was 0.995 (Spearman, p< 0.0001). The median of the differences was -1.67% and the range was -21.7% and +20.5%. **Conclusion:** The LC-MS/MS method was successfully validated and are able to perform a quantitative analysis of MTX levels in human blood and applied in clinical routine.

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Development and Validation of a Multiplexed LC-MS/MS Method for the Quantification of Biktarvy® (Bictegravir/Emtricitabine/Tenofovir Alafenamide) in Human Plasma

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Background: Human immunodeficiency virus (HIV) remains a public health concern, with an estimated global incidence of 1.8 million new infections per year. Antiretroviral therapy (ART) is the primary modality for treatment of HIV, and use of ART has decreased morbidity and mortality on the individual and population level. Biktarvy® (200 mg bictegravir (BIC)/200 mg emtricitabine (FTC)/25 mg tenofovir alafenamide (TAF)) is a fixed-dose drug formulation recently approved by the United States Food and Drug Administration (FDA) for HIV treatment. Although these drugs have shown therapeutic efficacy in clinical practice, treatment success can be compromised due to non-adherence, drug-drug interactions, or the propagation of drug-resistant HIV. Therefore, bioanalytical assays for drug quantification can prove beneficial in the assessment of drug efficacy and adherence. Thus, we have developed and validated a multiplexed liquid chromatographic–tandem mass spectrometric (LC-MS/MS) method for the quantitation of BIC, FTC and TAF in human plasma. **Methods:** BIC, FTC, and TAF, along with their respective isotopically-labeled internal standards, were extracted from human K_2EDTA plasma via solid-phase extraction. Post-extracted samples were analyzed using an API 4500 (SCIEX, Redwood City, CA) mass analyzer interfaced with a Nexera XR LC (Shimadzu, Kyoto, Japan). Antiretroviral agents were separated on a Waters Cortecs C18 column (2.7 µm, 2.1 x 50 mm) using a mobile phase system comprised of acetonitrile and water containing 0.5% formic acid. Analytes were measured in positive ionization and selective reaction monitoring (SRM) modes over an analytical run time of 6.0 minutes. The assay was validated in accordance with FDA Bioanalytical Method Validation, Guidance for Industry, recommendations. **Results:** For all drugs, calibration curves were generated using linear regression with $1/x^2$ weighting. Primary linearities were established as 5.00-10,000 ng/mL, 2.50-5,000 ng/mL, and 0.500- 5,000 ng/mL for BIC, FTC, and TAF, respectively. Inter-assay precision and accuracy was assessed at the lower limit of quantitation, and low, mid and high QC levels, and ranged from 3.9% to 13.5% and -8.2% to 14.1%, respectively. Dilutional studies demonstrated that specimens can be diluted two-fold and accurately measured; samples above the upper limit of quantitation were also diluted and accurately analyzed. Quantitative matrix effects were performed and showed average ion suppression of 18.5%, 6.9%, and 15.4% for BIC, FTC, and TAF, respectively; relative matrix effects were negligible. Carryover and crosstalk (analyte and internal standard) were not observed. Stability studies and application of the method to clinical trial samples are ongoing. **Conclusion:** We have developed and validated a dynamic LC-MS/MS method for the multiplexed measurement of BIC, FTC, and TAF in human plasma. This method has been validated in accordance with FDA recommendations, and is being validated in study participants on Biktarvy for HIV management.

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A Fast, Accurate, Precise Method for Free Valproic Acid on an Automated Chemistry Analyzer

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Background: Valproic acid is an antiepileptic drug approved by the FDA for the treatment of seizures, mania, and migraine disorders. Valproic acid is approximately 90% bound to albumin. The free portion of valproic acid is the pharmacologic active form

in the blood. The amount of valproic acid bound to protein can have high individual variation due to hypoalbuminemia, saturated protein binding, or co-medications. Therefore, this method was developed to measure free valproic concentrations to better assess therapeutic levels and possible toxicity. The development was performed on a chemistry analyzer, so it may be performed in a core laboratory to expedite turnaround time. **Methods:** The free valproic acid assay was developed on an automated chemistry analyzer (c502, Roche Diagnostics) by modifying a commercially available total valproic acid assay (VALP2, enzyme immunoassay, Roche Diagnostics). In order to achieve the needed sensitivity, the sample volume was modified from 2µL to 8µL. Six levels of calibrators (COBAS FP Free valproic acid Calibrators, Roche Diagnostics) with valproic acid concentration of 0.0, 5.0, 10.0, 15.0, 25.0 and 35.0 µg/mL were used for assay calibration. Separation of protein bound and non-protein bound fractions of valproic acid was achieved by ultrafiltration through a molecular weight cut-off filter (30 kDa, Millipore). The following assay performance characteristics were established; analytical specificity, analytical measurement range, analytical sensitivity, precision, accuracy, mixing study, dilution study, carryover, stability, and filtrate stability. **Results:** Free valproic acid was linear from 1.25 to 40.0 µg/mL with analytical recoveries ranging from 89.3 to 99.3%. Both intra- and inter-assay precision had coefficient of variations <5.0% at concentrations of 4.0 and 30.0 µg/mL. Forty samples were compared to the Integra 800 free valproic acid method (Roche Diagnostics). The correlation coefficient (R) was 0.9951, slope was 0.993, and intercept was -0.46. A mixing study was performed to determine an appropriate matrix for dilutions in order to extend the clinical reportable range. Saline was an acceptable matrix with percent differences <11%. A 1:2 dilution with saline was found to be acceptable with percent difference <5.0%. No carryover was observed up to the spiked concentration of 150.0 µg/mL. Stability in serum removed from cells was determined to be 5 days at 2 to 8 °C and 2 weeks at -20 °C. Filtrate was found to be stable for 1 hour at room temperature when left open to the air. **Conclusion:** This validated method on an automated chemistry analyzer provides fast, accurate, and precise results for the therapeutic drug monitoring of those patients administered valproic acid.

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Development of a High Throughput LC/MS-MS Method for Quantitative Detection of Two Phosphatidylethanol Homologues in Human Blood

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Background: Alcohol use disorder remains a significant health challenge in the United States affecting approximately 14.8 million people. Blood alcohol concentration (BAC) in serum/plasma or in breath is often used to assess acute alcohol consumption; however, BAC has a narrow detection window (< 12 hours). The non-oxidative ethanol metabolites, ethyl glucuronide and ethyl sulfate, are direct biomarkers of ethanol exposure with a detection window of 2 - 5 days in urine. Traditional long-term biomarkers of chronic alcohol consumption like mean corpuscular volume, gamma-glutamyltransferase, aspartate aminotransferase, and alanine aminotransferase lack adequate specificity and can be elevated by other etiologies. Therefore, a long-term, specific, and direct biomarker of alcohol consumption is desirable. Phosphatidylethanol (PEth) is an ethanol-derived phospholipid produced from phosphatidylcholine through a transphosphotidylation reaction catalyzed by phospholipase D. Once produced, PEth is incorporated into erythrocyte cell membranes and can be detected for 2 - 4 weeks following ethanol consumption. PEth is a group of ~48 glycerophospholipid homologs due to variations in the fatty acid moieties attached to the phosphoethanol head group. The predominant PEth homologues are PEth 16:0/18:1 (POPEth) and PEth 16:0/18:2 (PLPEth), which accounts for 37 - 46% and 26 - 28% of the total PEth homologues, respectively. In this study, we describe the development and validation of a selective, robust, and high throughput LC/MS-MS method for quantifying POPEth and PLPEth in human blood. **Methods:** Fifty µL of internal standards (POPEth-d5 and PLPEth-d5) was added into 100 µL aliquot of whole blood samples and vortexed. Protein precipitation was achieved using a 10% (v/v) isopropanol (IPA) / acetonitrile (ACN) solution. Upon centrifugation, the supernatant was extracted and buffered with 15 mM ammonium acetate. Sample extracts were analyzed by LC-MS/MS using an Agilent 6470 triple quadrupole mass spectrometer coupled to an Agilent LC system equipped with two 1260 Infinity II binary HPLC pumps, a 1260 Infinity II autosampler, and a 1260 Infinity II thermostatted column compartment. The mass spectrometry method was performed on a negative ion electrospray with MRM acquisition. Chromatographic separation was achieved using a Phenomenex Luna Omega Polar C18 column (100A, 1.6 µm 2.1 x 50 mm) maintained at 60 °C on a 0.5 mL/min gradient elution consisting of 5 mM ammonium acetate in 30/70 H_2O/ACN (mobile phase A) and 5 mM ammonium acetate in 30/70 IPA/ACN (mobile phase B). Automated alternating column regeneration was used to increase the chromatographic throughput. Analytical validation of the LC-MS/MS method was performed in whole blood for linearity, accuracy, imprecision, sensitivity, specificity and ion suppression/matrix effect, in accordance to CLSI guidelines. **Results:** The analytical measurement range, 10 - 2000 ng/mL, was linear with R^2 of 0.999 for both PLPEth and POPEth. The within-run and total imprecision was < 5% CV for the low (20 ng/mL), medium (200 ng/mL) and high QC (1000 ng/mL). Results from accuracy and method comparison experiments met the bias criteria of ±20%. **Conclusion:** The LC-MS/MS method described in this study for detection of POPEth and PLPEth in human blood showed adequate analytical performance as a sensitive and specific assay for evaluating long-term alcohol consumption.

B-287

Effect of Dose Individualization Based on Therapeutic Drug Monitoring Consultation on Vancomycin Treatment

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Background: Appropriate dosing of vancomycin is crucial in the adequate control of the source of infection and in preventing the development of antimicrobial resistance and toxicity. However, inter- and intrapatient variability in pharmacokinetics make it difficult to achieve therapeutic vancomycin concentrations. This study aimed to determine the clinical effect of therapeutic drug monitoring (TDM) consultation on vancomycin treatment. **Methods:** Among 2,222 patients who underwent vancomycin TDM over one year period, 528 patients (24%) received TDM consultation. TDM consultation was provided with Bayesian forecasting for dose individualization using Abbott PKS software. We selected 276 adult patients (>18 years) hospitalized in surgical department for evaluation of the effect of TDM consultation for vancomycin treatment. We compared baseline clinical characteristics and laboratory findings between the patients who received TDM consultation (Group 1) and who did not (Group 2), by a retrospective review of the electronic medical record. **Results:** The results of the comparison between the two groups are summarized in Table 1. There was no significant difference in demographics, vancomycin indication and baseline serum creatinine level between the two groups. However, Group 1 showed a significantly longer duration of vancomycin therapy and a higher rate of concomitant use of a nephrotoxic agent. Moreover, these patients showed a significantly higher occurrence rate of nephrotoxicity and maximum vancomycin concentration. Nevertheless, there was no significant difference in the final vancomycin concentrations between the two groups and the rate of achieving therapeutic level was significantly higher in Group 1. The compliance rate (change of dose regimen according to consultation) to TDM consultation was 83% (115/139).Table 1.

	Group 1(with TDM consultation)	Group 2(without TDM consultation)	*p* value
No. of patients	139	137	
Age, median (range), yearsGender, male, n (%)	67 (26-85)89 (64.0%)	63 (23-89)75 (54.7%)	NSNS
Weight, median (range), kg	61.0 (35.9-96.7)	63.1 (40.6-96.4)	NS
Vancomycin treatment indication, n (%)			
Skin, skin structure & soft tissue infectionsBacteremia, sepsisSurgical infection prophylaxis	52 (37.4%)16 (11.5%)3 (2.2%)	35 (25.5%)16 (11.7%)10 (7.3%)	NSNSNS
Length of vancomycin therapy, median (range), days	8 (1-62)	5 (1-26)	$p < 0.0001$
Concomitant use of nephrotoxic agent (yes), n (%)	85 (61.2%)	66 (48.2%)	$p = 0.0406$
Occurrence rate of nephrotoxicity, n (%)	25 (17.9%)	10 (7.3%)	$p < 0.0001$
Maximum vancomycin concentration, median (range), µg/ml	19.0 (3.1-42.8)	16.8 (2.8-38.9)	$p = 0.0003$
Final vancomycin concentration, median (range), µg/ml	15.9 (3.1-31.8)	14.7 (2.8-38.1)	NS
Therapeutic concentration, n (%) Sub-therapeutic concentration, n (%) Supra-therapeutic concentration, n (%)	100 (77.5%)19 (14.7%)20 (15.5%)	76 (55.5%)37 (27.0%)24 (17.5%)	$p = 0.0001$ $p =$ 0.0214NS

Conclusions: Although patients who received TDM consultation had higher risk of vancomycin-induced nephrotoxicity at baseline, they showed a higher rate of achiev-

ing therapeutic vancomycin concentrations. This finding suggests that dose adjustment based on TDM consultation could help to increase the safety and effectiveness of vancomycin treatment.

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Comparison of a New Vitamin k1 LC-MS/MS Method for Serum Dosage with HPLC Reference Method: Results and New Reference Range

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Background: Vitamin K1, or phylloquinone, is part of multiple similarly structured fat-soluble molecules containing a 2-methyl-1,4-naphthoquinone ring structure called menadione. Major sources of Vitamin K1 include spinach, cabbage, and kale. Vitamin K1 is the essential cofactor for the carboxylation of glutamic acid residues in many vitamin K-dependent proteins (VKDPs) that are involved in blood coagulation, bone metabolism, prevention of vessel mineralization, and regulation of various cellular functions. Measurement of vitamin K1 in fasting serum is a strong indicator of dietary intake and status. **Objective:** Validate the new LC-MS/MS method for vitamin k1 dosage in serum for dietary status by comparison with HPLC reference method and generate a new reference range. **Methods:** The analysis was performed by LC-MS/MS Waters XEVO TQ-S micro with electrospray ionization (ESI+) after a protein precipitation and liquid-liquid extraction. In total, 50 samples of volunteers were analyzed, and from these samples, 30 were analyzed in both methods. The methods were compared by Passing-Bablok regression and Bland-Altman analysis (Figure 1). The reference range were generated by indicated CLSI protocol for transferring reference ranges. **Results:** The methods showed a good correlation: 0.982 (CI95%: 0.962-0.992; p<0.0001-Spearman) and slope of 0.7280 (CI95%: 0.6625-0.8333) and interception of +10.26 (CI95%: -15.4 to +30.7). The HPLC method had a reference range between 80-1160 pg/mL. We obtained the range between 68 (CI95%: 37-98) to 855 (CI95%: 753-997) pg/mL. Overall, 49/50 samples of normal volunteers was contained in this range, except one sample (1919 pg/mL), as considered an outlier (Dixon test) and it was kept in the analysis. **Conclusion:** Although there are a significant difference between the methods with average lower results of 21.1% for LC-MS/MS method, the new interval derivate from HPLC method used in another laboratory was successfully implemented. This was possible due the excellent correlation between the methods.

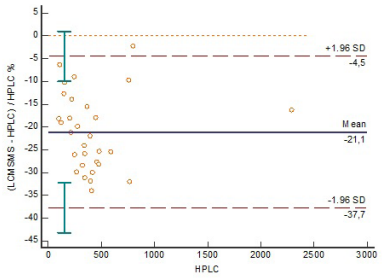

Figure 1. Bland-Altman plot for the 30 samples analyzed by HPLC and LC-MS/MS methods.

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Assessment of Circulating miR-20b, miR-221 and miR-155 in Occupationally Cadmium Exposed Workers of Jodhpur, India

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Background: Cadmium (Cd), a well-known environmental pollutant, can induce several adverse health effects following its accumulation in the human body. Cadmium is also a potential carcinogen, capable of causing multi-system alterations. The molecular mechanisms by which cadmium exerts systemic damage in the human body are under exploration. Recent reports in cell lines do report an association of immune alteration, DNA damage, altered DNA repair gene expression in post-chronic cadmium exposure. Cadmium is also known to alter DNA methylation as a part of epigenetic regulation. Heavy metals are also known to alter miRNA, small non-coding RNA molecules capable of controlling gene expression, but there has been no reported study of miRNA alteration in cadmium exposure. The present study had an aim to assess the circulating levels of miRNA-20b, 221 and 155 in occupationally cadmium exposed workers and correlate them with blood lead levels.

Methods: 107 participants working in wielding factory of Jodhpur, India and 96 apparently healthy participants without a history of occupational Cadmium exposure were recruited after obtaining informed consent. Blood Cadmium level was estimated by graphite furnace atomic absorption spectrophotometry (GF-AAS). Circulating miRNAs were isolated from serum by Qiagen miRNA isolation kit and converted to cDNA by Qiagen LNA RT kit. Expression profiles of miR-20b, miR-221 and miR-155 was performed in RT-PCR (Biorad CFX96) using specific Qiagen miRNA PCR assays. miR-16 was used as an endogenous control. Qiagen Spike-In kit was used as exogenous control. The miRecords database, an integration platform of miRNA target prediction was used to predict the target genes of miRNAs. Functional analysis of these predicted target genes was done in STRING database and KEGG database Statistical analysis was done in GraphPad Prism 8. The normality of data distribution was assessed using the Shapiro-Wilk test. Mann-Whitney U test was used to compare the differences among the two groups. A p-value<0.05 was considered statistically significant.

Results: The blood cadmium level (mean± SD) of occupationally exposed subjects was significantly higher (2.4± 1.2 µg/l) in comparison to the non-exposed group (1.0± 0.7 µg/l) (p<0.05). Among the three miRNAs, only miR-221 had significant upregulation of 2.63-folds in the exposed group (p<0.05). The fold changes of miR-155 and miR-20b among two groups were 1.62 and 1.07 respectively. Functional analysis revealed that miR-221 has the potential to trigger various cellular genes and pathways.

Conclusion: The findings of our study highlight the importance of miRNA dysregulation in cadmium exposed individuals that may contribute to the systemic effects of cadmium toxicity. The role of miR-221 and other miRNAs as a potential biomarker of cadmium toxicity needs to be further validated in diverse population groups to highlight the possible molecular pathways through which Cadmium exerts its toxic manifestations.

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Is Ceruloplasmin as a Copper Indicator Still Viable?

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Background: Copper is an essential metal present in various biological processes and bound to metalloproteins. Their intake ranges from 1 to 1.6 mg / day (minimum daily requirement of 0.9 mg) and the main source is cereals. Absorbed between 20 and 50% in the intestine and transported bound to albumin. Stored in the liver (ceruloplasmin) and excreted in bile, feces and urine. It participates in several biological functions: energy production; in connective tissue; Metabolism of Fe; CNS and anti-oxidant.

Methods: The present study aims to evaluate ceruloplasmin as the indirect indicator of copper levels A retrospective survey was performed on the results of plasma copper and ceruloplasmin, analyzed in the laboratory routine of patients, performed between July 2016 and July 2017. Plasma copper concentrations were quantified by atomic absorption spectrophotometry and ceruloplasmin was determined by nephelometry

Results: A total of 4,193 cases were performed, which performed concomitant ceruloplasmin and copper analysis. We found 655 (15.6%) cases of copper altered and 390 (9.3%) of ceruloplasmin. Ceruloplasmin levels (high, normal and low) were analyzed as follows: normal ceruloplasmin does not detect 9.2% of altered copper (high and low); Ceruloplasmin high in 1.7% of cases erroneously classified normal copper as high; And in 38% of cases low ceruloplasmin classified normal as low

Conclusion: despite its low cost and analytical ease, ceruloplasmin does not perform compatible with plasma copper dosage

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Discovery of novel medication interferents on point-of-care urine drug screen assays through analysis of electronic health record data

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Background: Point-of-care assays allow clinicians to quickly receive a result that they can use to inform patient care. Like all drug screening immunoassays, the point-of-care urine drug screen (UDS) assays are susceptible to false-positive results. Most laboratory best practice guidelines recommend positive screens be followed by mass spectrometry-based confirmatory testing. Confirmation testing can take days to weeks, which is incompatible with immediate use of results at the point of care. Due to the rapid incorporation of point-of-care UDS results into patient care, it is essential that providers be aware of substances that may cause false-positive screening results. At present, a complete list of substances that can cause false-positive results is not

available. We took a statistical approach, previously used to discover interferences on automated chemistry UDS, to find new cross-reacting medications on the point-of-care UDS assays.

Methods: Our institution uses a point-of-care UDS immunoassay that contains all the reagents necessary to screen for methamphetamine, amphetamine, benzodiazepines, barbiturates, opiates, cocaine metabolites, methylenedioxymethamphetamine (MDMA), cannabinoids, methadone, oxycodone, propoxyphene, and buprenorphine (Integrated E-Z Split Key Cup II, Alere®). We extracted paired point-of-care UDS and reflexed confirmation results from our institution's de-identified electronic health record (EHR) database. We defined a false positive screen as a positive screen result followed by a negative confirmation result. We identified medication exposures occurring 1-30 days before each patient's UDS result. We mapped each medication to its active ingredient(s) using RxNorm, creating a list of the distinct ingredients to which a patient was exposed in the 30-day period before the UDS. We used Firth's logistic regression to quantify the associations between exposure to particular ingredients and false-positive UDS results, which yielded odds ratios (OR), 95% confidence intervals, and p-values. We then validated cross-reactivity by spiking compounds into drug-free urine at various concentrations (0, 50, 100, 200, 400, 800 and 1600 mcg/mL) and testing the urine on the point-of-care UDS assay.

Results: Our EHR dataset included 120,670 UDS results: 110,604 negatives, 9,620 true-positives, and 446 false-positives. Despite having fewer false-positive results in this dataset than in our previous study, our statistical analysis pointed to the following potential cross-reactivities: ranitidine for methamphetamine (OR=8.6), prazosin for methadone (OR=9.9), labetalol for MDMA (OR=70.9), and meloxicam and furosemide for cannabinoids (OR=73.6 and 12.9, respectively). Upon experimental validation, ranitidine produced positive screening results for methamphetamine at 50 mcg/mL, morphine at 1600 mcg/mL, and propoxyphene at 800 mcg/mL. Labetalol produced positive screening results for MDMA at 800 mcg/mL. Prazosin, furosemide, meloxicam and metabolite 5'-carboxy-meloxicam and did not produce positive results up to the highest concentration tested (800 mcg/mL for 5'-carboxy-meloxicam, 1600 mcg/mL for remainder).

Conclusion: The analysis of data from the EHR successfully returned a list of potential cross-reacting compounds on the point-of-care UDS from a limited dataset of only a few hundred false-positive results. Two out of the five potentially cross-reacting compounds validated experimentally to cause false-positive results. These findings support the generalizability of our approach to identify medications that interfere with clinical immunoassays, as well as the utility of EHR data for advancing laboratory medicine.

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Performance Evaluation of ChromSystems™ LC-MS/MS Drugs of Abuse Test Kit for Simultaneous Detection of Multiple Drugs and Metabolites in Urine

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Background: Urine drug testing (UDT) in clinical settings is often used to assess presence of prescription or illicit drugs. Conventional immunoassay-based drug screening is limited by the number of available drugs and assay cross-reactivity. As a result, an additional step for identification and confirmation by GC-MS or LC-MS is often necessary. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a rapid method, which is highly suitable for simultaneous detection of multiple analytes with high sensitivity and specificity. Although MS-based testing has seen an enormous growth in the clinical laboratory testing and considered to be a "gold standard" in UDT, it is typically an in-house developed test, which requires extensive optimization and validation. The availability of commercial UDT kits can help laboratories to overcome this challenge. The purpose of this study was to evaluate analytical performance of commercially available drugs of abuse kit on UPLC-MS/MS platform for targeted detection of common drugs and metabolites in urine samples. The ChromSystems™ reagent kit was evaluated for confirmatory testing of 99 substances, including amphetamines, benzodiazepines, cocaine, cannabinoids, opioids, Z-drugs, hallucinogens and "boosters".

Methods: Sample preparation used 50 µL of urine, 10 µL of internal standard, and 40 µL of glucoronidase solution. Isotope-labelled internal standards are used for the majority of analytes, as well as few structurally related co-eluting compounds. Following enzyme hydrolysis (2h, 45°C), 100 µL of the precipitation buffer was added, the sample was centrifuged (5min, 15,000×g) and 100 µL of supernatant was diluted with a buffer prior to analysis. Sample was eluted *via* gradient elution at a flow rate of 0.4 mL/min on Waters Acquity UPLC, ionized by electrospray ionization in positive mode (ESI+) and analyzed by XEVO tandem quadrupole MS by mixed reaction monitoring (MRM). The quantifier and qualifier MRM transitions were measured for each analyte and a single transition was measured for each internal standard. The assay run time was 12.5 min. Standard validation for within day and between-day precision, limit of detection (LOD), limit of quantification (LOQ), and linearity were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Majority of analytes exhibited good linearity with a coefficient of determination >0.98 over the concentration ranges investigated. Assay linearity was determined for each analyte using six-point calibration curves. The lower limits of detection (LLOD) and lower limits of quantification (LLOQ) for majority of analytes were determined to be in the range 1-8 ng/mL and 1-20 ng/mL (CV<20%), respectively. Precision measured on twenty separate days for low, medium, and high QC samples were <10% for over 80 analytes and nine analytes exhibited CV>20%. Further validation and optimization of specific analytes (*i.e.* buprenophrene, norbuprenophrene, bromazepam, brotizolam, hydroxyimidazolam, hydroxybromazepam, clonazepam, desalkylflurazepam, LSD) is currently underway.

Conclusion: The LC-MS/MS method based on commercially available kit for UDT is sensitive and provides easy to follow steps for sample preparation. Preliminary data indicates it has satisfactory clinical performance for the majority of tests at recommended cut-off values. On-going optimization of MRM transitions and ion source parameters could further improve performance of the entire drug panel.

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Positivity Rates for Urine Toxicology Testing Performed at a Large University Medical Center

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Introduction: Screening for presence of drugs of abuse as well as for compliance with therapeutics in pain management is increasingly being performed by clinical chemistry laboratories. We have recently developed an in-house LCMSMS-based testing to complement an immunoassay-based screening to support growing clinical needs. The LCMSMS-based testing was initially developed for opiates and gradually expanded to include amphetamines, benzodiazepines, cocaine metabolites, and cannabinoids. In this study, we assessed the utility of the service by examining positivity rates for available tests menu.

Methods: All urine toxicology results obtained by LCMSMS methodology that were reported between July 2016 and December 2019 were obtained and analysed for individual tests volume and for positivity rates over the study period.

Results: Testing volume rapidly increased from 6 samples in year 2016 to 87 samples per month in year 2019 for opiates confirmation. Request for opiates confirmation increased by 44%, and 67% for the years 2018, and 2019 respectively. Similarly, for amphetamines and for benzodiazepines tests volume increased by 54.5% and by 53.7% from 2018 to 2019 respectively. Positivity rates for major opiates (hydromorphone, morphine, hydrocodone) were at 74%, 29%, 69% (2016); 68%, 42%, 72% (2017); 56%, 52%, 47% (2018); and 53%, 50%, 46% (2019) respectively. Very low positivity rates were observed for triazolam at less than 1%, diazepam and midazolam at below 6%, MDMA/MDA below 2%, and phentermine less than 14%.

Conclusion: Although the positivity rates reported reflect our patient population and clinical practices, the high positivity rates for most tests suggest appropriate utilization and support of clinical needs. The few tests with low positivity rates such as diazepam and MDMA suggest that a review for its continued availability is needed. Future studies will examine the impact on outcomes and on the need for targeted test order sets and menu availability.

B-296

Method Comparison of Seven TDM Drugs across Three Abbott Diagnostics Platforms: Architect c, Architect i, and Alinity c

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Background: Therapeutic drug monitoring (TDM) is essential for several drugs with a narrow therapeutic range, where serum levels can often become toxic (too high) or ineffective (too low). Abbott Diagnostics currently offers TDM drug assays on three platforms: Architect c, Architect i, and Alinity c, although a three-way method comparison has yet to be performed. Therefore, we compared TDM drug assays, including antiepileptic drugs (AEDs), antibiotics, a cardiovascular medication, and a respiratory medication across these three Abbott Diagnostics analytical platforms. **Methods:**

Method comparison was performed for seven TDM drugs (AEDs (phenobarbital, phenytoin, valproic acid), antibiotics (gentamicin, vancomycin), a cardiovascular medication (digoxin), and a respiratory medication (theophylline)) across three automated analyzers: Architect c, Architect i, and Alinity c (Abbott Diagnostics) at the University Health Network (Toronto, Canada). TDM drug concentration (n=40-50 samples/drug) was compared using Pearson correlation, Deming regression (including assessment of residuals), absolute bias, and percent bias. Imprecision was assessed for all TDM drugs by measuring three levels of quality control (QC) material (Technopath Multichem IA plus) in duplicate twice per day for 5 days. Acceptable imprecision and bias were determined based on CLIA recommendations. **Results:** All TDM drug assays correlated well across all platform comparisons (i.e. r^2 varied between 0.94-1.00). Architect c versus Alinity c exhibited a strong correlation (r^2>0.95) and minimal bias (<12.5%) for all TDM drug assays. For Architect c versus Architect i, a strong correlation, minimal bias, and Deming regression slopes close to 1 (i.e. 0.96-0.99) was achieved for all TDM drugs except phenytoin. Phenytoin exhibited a positive bias of 16.0%, although it exhibited a strong correlation (r^2 = 0.98) and Deming regression slope of 1.072. Alinity c versus Architect i showed a strong correlation, minimal bias, and Deming regression slopes close to 1 (i.e. 0.805-1.029) for all TDM drugs except digoxin. Digoxin exhibited a positive bias for Alinity c versus Architect i (15.9%), although it exhibited a strong correlation (r^2 = 0.963) and Deming regression slope of 1.06. Importantly, the positive bias observed for digoxin was only apparent at low concentration levels (i.e. <0.7 ng/mL). Imprecision for all TDM drug assays was within CLIA recommendations across all QC levels on all analyzers. **Conclusion:** All TDM drug assays analyzed exhibited acceptable comparability across all three Abbott platforms (i.e. correlation and bias) except for phenytoin (positive bias on Architect c versus Architect i) and digoxin (positive bias on Alinity c versus Architect i). Furthermore, the imprecision of all TDM drug assays were within CLIA recommendations for all QC levels and analyzers. When changing platforms for TDM monitoring in a clinical laboratory, it is important to perform an extensive validation and communicate any expected changes with physicians.

B-299

Here Today, Gone Tomorrow - Evaluating the Stability of Bupropion and Chlorpromazine in Serum/Plasma

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Background: Bupropion (Wellbutrin®, Zyban®) is an antidepressant drug, indicated for the treatment of major depressive disorder and it is also used as an aid for smoking cessation. Chlorpromazine (Thorazine®) is an antipsychotic drug that is prescribed to treat psychotic disorders, such as schizophrenia and manic-depression in adults. Therapeutic drug monitoring for bupropion and chlorpromazine is used to optimize drug therapy, evaluate compliance, and assess adverse effects. This study describes the development and validation of an assay for the quantification of bupropion, its metabolite hydroxybupropion, and chlorpromazine in serum/plasma using solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Methods:** An internal standard solution consisting of the deuterated analogue for each analyte was added to an aliquot of calibrators, quality controls, and patient serum or plasma. Analytes were separated from the biological matrix using SPE. Dried extracts were reconstituted in mobile phase and separated by liquid chromatography using 5 mM ammonium formate, 0.05% formic acid in water and 0.1% formic acid in acetonitrile mobile phases and a Kinetex F5 column. Eluate was introduced into an Agilent 6470 triple quad mass spectrometer, via positive mode electrospray ionization (ESI), utilizing multiple-reaction monitoring (MRM). Analyte quantification was performed using the Agilent MassHunter Quantitative Analysis software. **Results:** Method validation experiments were performed for linearity, accuracy, imprecision, sensitivity, specificity, stability, carryover, and ion suppression/matrix effect, in accordance to CLSI guidelines. The analytical measurement range (AMR) was 5-450 ng/mL for bupropion, 50-1500 ng/mL for hydroxybupropion, and 25-750 ng/mL for chlorpromazine. Total imprecision (%CV) was < 10% for three quality control concentrations for each analyte. Accuracy studies were performed by spiking individual negative samples with known concentrations for each drug and metabolite. Accuracy results met the acceptance criteria of +/- 15% of target. The quantification of bupropion and hydroxybupropion was not prone to interference from high concentrations of hemoglobin, triglycerides, and bilirubin. However, hemoglobin >13 mg/dL was found to suppress chlorpromazine beyond acceptable limits. During method validation, it was discovered that bupropion was not stable at ambient temperature for more than 6 h and was stable for 24 h at refrigerated temperature, yet steadily degraded thereafter. Instability of this analyte complicated method comparison with an external laboratory. Hydroxybupropion did not exhibit limitations in stability at ambient (6 h), refrigerated (14 days), or frozen temperatures (14 days). Chlorpromazine was not stable at ambient temperature for 6 h, yet had at least 14 days stability at refrigerated temperature. The analytical method and results from method validation experiments will be described in detail in the poster. **Conclusion:** A LC-MS/MS method was developed and validated, according to CLSI guidelines, for the quantification of bupropion, hydroxybupropion, and chlorpromazine in patient samples for serum and plasma. Stability studies revealed that patient specimens must be immediately stored frozen until analysis to preserve the concentration of bupropion. Patient samples for chlorpromazine must be stored refrigerated or frozen.

B-301

Development of a Simple and Sensitive Method for Accurate and Reproducible Blood Lead Measurement on Single and Triple Quad Inductively Coupled Mass Spectrometers

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Background: Lead toxicity is predominately found in children age 6 months to 5 years, however it is not limited to this group. Primary sources of lead exposure include lead-based paints, airborne lead, occupational sources, food and drinking water, and lead glazed pottery. Lead toxicity largely effects three systems: nervous, hematopoietic, and renal. Although a sector of the population remains at high risk of exposure, blood lead concentrations have declined dramatically in the US in the last 2 decades. The 97.5th percentile in children 1-5 years old is approximately 3.5 µg/dL, which is lower than the current reference value of 5.0 µg/dL. This decline requires lead assays that can accurately and reproducibly measure low lead concentrations, and tighter acceptability criteria for analytical errors. Our objective was to develop and validate a simple, sensitive, reproducible and accurate assay for the quantification of lead on both a single and triple quad inductively coupled mass spectrometer (ICP-MS), following the Advisory Committee on Childhood Lead Poisoning Prevention (ACCLP) analytical criteria of ±2 µg/dL or ±10%, whichever is greater. **Methods:** This method was developed on a Thermo Fisher iCAP RQ and TQ ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) in kinetic energy discrimination (KED) mode. The method was calibrated using a certified reference material traceable to NIST SRM 3128 (VHG Labs, Manchester, NH, USA). Calibration was verified using a reference standard solution traceable to NIST CRM 3128 different than the calibrator (Inorganc Ventures, Christianburg, VA, USA). Briefly, EDTA whole blood (50 µL) was added to 0.5% nitric acid (9.95 mL), vortex mixed and centrifuged for 5 minutes at 3,500 g. Samples were loaded on an Elemental Scientific Inc. (ESI, Omaha, NE, USA) SC-FAST sample introduction system. Total cycle time is 45 seconds and 65 seconds on the RQ and TQ respectively. The following assay performance characteristics were established; analytical measurement range, analytical sensitivity, imprecision, accuracy, mixing study, and carryover. **Results:** A 1:1 mixing study was performed using 10 different patient samples to determine an appropriate matrix for dilution and calibrator preparation. Nitric acid (0.5%) was determined to be an acceptable matrix with a mean percent bias of 4.2%. Lead was linear from 1.0 to 100.0 µg/mL with analytical recoveries of certified reference material ranging from 89.5 to 102.2%. The limit of blank, limit of detection, and limit of quantitation were determined to be 0.008 µg/dL, 0.074 µg/dL, and 0.92 µg/dL respectively. Precision at the LOQ showed coefficient of variations <6.0%. Both within run and between day imprecision were acceptable with total coefficient of variations <3.1% at concentrations of 6.0 and 22.0 µg/dL. Forty sample, across the measurement

range, were compared to a previously validated in-house method. The correlation coefficient (R) was 0.9994, slope was 1.006, and intercept was -0.02. Comparison between the RQ and TQ systems showed the correlation coefficient (R) was 0.9999, slope was 1.085, and intercept was -0.06. No carryover was observed up to 115.5 µg/dL. **Conclusion:** This validated ICP-MS method offers simple, sensitive, reproducible and accurate quantification of lead on both ICP-MS systems.

B-302

Development and Validation of LC/MS/MS Method for Qualitative Drug Testing of Umbilical Cord Tissue

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Background: Umbilical cord tissue (UCT) drug testing may be helpful for detection of drug exposure *in utero*. Detecting drug exposure enables early medical intervention to reduce risks, such as Neonatal abstinence syndrome (NAS) associated with maternal drug use during pregnancy. NAS is a disorder characterized by symptoms

of drug dependence and withdrawal. Opioid use, in particular, during pregnancy is associated with NAS occurrence. The January 11, 2019 CDC *Morbidity and Mortality Weekly Report* reported an increase of 433% in the incidence of NAS in US from 2004 to 2014, from 1.5 to 8.0 per 1,000 hospital births. Since our hospital recently started the labor delivery service, we would offer UCT drug testing. The objective of this study was to develop a highly specific and sensitive Liquid Chromatography Mass Spectrometry method to qualitatively detect 41 prescription including opioids and amphetamines, and non-prescription drugs and their metabolites such cocaine metabolite in human UCT and correlated results with Ohio State University's Department of Pathology Laboratory. **Methods:** UCT samples were homogenized using a Biotage Lysera™ homogenizer and centrifuged for ten minutes. Enzymatic hydrolysis was performed by adding IMCZyme™ β-Glucuronidase, with heating in a water bath. After centrifugation, samples were extracted by mixed mode solid phase extraction (SPE) utilizing cation exchange and reverse phase interaction (Biotage Evolute Express CX). During SPE cleanup, samples washing included: phosphate buffer/acetic acid, and water/methanol/acetic acid. Samples were eluted with ethyl acetate/hexane and dichloromethane/methanol/ammonium hydroxide. Eluents were evaporated to dryness using a Biotage Turbovap™ LV evaporator and reconstituted with water/acetonitrile/formic acid. Using a Shimadzu Nexera™ liquid chromatograph system and Sciex Citrine™ 6500 mass spectrometer, samples were qualitatively detected for analytes of interest. Chromatographic separation was carried out using a Restek Raptor™ biphenyl column. An eight-minute gradient elution was performed. Mobile phases were: formic acid in water and formic acid in acetonitrile. Mobile phase flow rate was 0.6 mL/min initially, and then increased to 0.8 mL/min after analyte elution. Analyses were performed in positive mode using scheduled multiple reaction monitoring (sMRM). **Results:** Cut-offs range for all drugs were between 0.1 and 2.0 ng/g. Free drug recoveries from glucuronide conjugates range were between 90 and 114%. Correlation with Ohio State University, performed by analyzing 12 samples, yielded 41 mutually detected positives for 17 drugs. **Conclusion:** The above on-site UCT drug testing would serve as an adjunct diagnostics, on a timely basis, for babies with suspected in utero drug exposures.

B-306

Impact of environmental chemicals on platelet bioenergetics and quality

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Background: Platelets are metabolically active cellular fragments that play critical roles in hemostasis. Changes in platelet metabolism and mitochondrial function have been linked to several metabolic and neurodegenerative diseases. In addition to contributing to the mechanism of these diseases, the loss of quality through mitochondrial dysfunction makes the platelets incapable of protecting against oxidative or metabolic stress. This suggests that the determination of metabolism and mitochondrial function in platelets have the potential to assess the quality of platelets. An important determinant of platelet function is environmental chemicals that reach the blood circulation through diet and medication. However, their impact on platelet bioenergetic function remains largely unclear. Recent advances that integrate metabolomics, bioenergetics and bioinformatics have allowed a comprehensive analysis of the impact of environmental pollutants on platelet quality and function. The objective of the study is to determine the impact of environmental chemicals on platelet bioenergetics and to test the utility of bioenergetic measurements in assessing platelet quality. It is hypothesized that the environmental pollutants that accumulate in platelets interfere with the metabolic and bioenergetic function and decrease quality.

Methods: Platelets collected from healthy donors have exposed to environments chemicals belong to the categories of pharmaceuticals, natural products, industrial chemicals, pesticides, herbicides, etc. at physiologically relevant concentrations. The mitochondrial function of the platelets was determined by mitochondrial stress test using extracellular flux analysis which measures cellular oxygen consumption and extracellular acidification that correspond to mitochondrial function and metabolism respectively. 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). In addition, mitochondrial respiratory enzyme complexes (Complex I, Complex II and Complex IV) are determined mitochondrial complex activity in selectively permeabilized platelets in the presence of specific metabolic substrates. Untargeted metabolomics was performed using previously established high-resolution mass spectrometry (HRM) methods. Metabolic features that are significantly altered were annotated using xMSannotator. Bioenergetic and HRM data from the same set of samples were integrated by using xMWAS.

Results: Metabolomics analysis of the platelets show that more than 40% of the metabolites that impact bioenergetic function are environmental pollutants. Furthermore, it has been identified that there are chemicals belong to the categories of pharmaceuticals, industrial chemicals, natural products and pesticides. Exposure of healthy platelets to these chemicals showed significant inhibition of the bioenergetic function, specifically maximal respiration and reserve capacity which suggests that environmental chemicals render platelet highly susceptible to bioenergetic dysfunction.

Conclusion: Taken together, these studies show that environmental chemicals accumulate in platelets and inhibit the mitochondrial function in platelets. It may also be concluded that platelet bioenergetics may serve as a test to assess platelet quality.

B-307

Trends in Fentanyl and Analogues Prevalence in Emergency Medicine Patients: A Retrospective Analysis Using High Resolution Mass Spectrometry

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Background: The death toll due to opioid overdoses has been increasing in the United States, with a particular spike in this trend caused by the emergence of synthetic opioids including fentanyl and analogues. There have been numerous reported cases of synthetic opioids as adulterants in heroin, methamphetamine,cocaine, and counterfeit pills. As a result, they are often times consumed unknowingly, causing mixed toxidromes and confounding diagnosis. Proper routine drug monitoring is essential in addressing the ever-expanding magnitude of the opioid epidemic. This study has two main objectives: 1) to observe prevalence of positive fentanyl screens among emergency medicine populations screened for drugs of abuse, along with trends for co-positives with other drug classes; 2) to identify fentanyl analogs andother false positives in these positive fentanyl screens in order to evaluate local trends in substance abuse. **Methods**: Analysis of remnant clinical samples was approved by the UCSF Institutional Review Board. Urine samples from emergency department (ED) patients at San Francisco General Hospital with drugs-of-abuse screens performed were monitored from October 2018 to March 2019 for presence of fentanyl(via EMIT immunoassay). Samples which were positive for the fentanyl screen were collected and further characterized by high resolution mass spectrometry (LC-HRMS). Urine samples were diluted 1:5 and chromatographic separation of analytes was performed using a Kinetex C18 column with a 10-minute gradient from 2%-100% organic. Data was collected on a SCIEX TripleTOF®5600 using a positive-ion mode TOF-MS survey scan with IDA-triggered collection of high resolution product ion spectra (20 dependent scans). Data review was done using PeakView and MasterView software. Data was screened for fentanyl, norfentanyl, 13 literature-reported synthetic opioids, and compounds reported/suspected to cause false positives with fentanyl immunoassays. **Results**: 158 emergency department patient samples screened positive via EMIT fentanyl immunoassay (7.44%of all ED samples screened for drugs of abuse). Amongst opiates positive patients, 13.21% screened positive for fentanyl. 8.26% of cocaine positive and 6.84% of amphetamines positive patients also screened positive for fentanyl. These samples were analyzed via LC-HRMS and analysis was targeted for fentanyl, norfentanyl, other 13 literature-reported synthetic opioids, and commonly prescribed or over-the-counter pharmaceuticals with chemical structures similar to that of fentanyl. 6.9% of samples were found to be positive for both fentanyl and acetyl fentanyl. Additionally, risperidone, its metabolite 9-hydroxyrisperidone (paliperidone), and loperamide with its metabolite desmethylloperamide were discovered as sources of false positive for fentanyl screens. **Conclusion**: This study reveals the non-trivial prevalence of fentanyl in the ED patient population being screened for drugs of abuse and emphasizes the importance of including fentanyl in drug screen panels. In many of the cases, due to current protocols, fentanyl was not suspected or discovered as exposure agent prior to more comprehensive testing or patient discharge. Additionally, identification of acetyl fentanyl along with fentanyl via HRMS analysis can hint to a changing trend in provenance of illicitly manufactured fentanyl. Finally, HRMS is a valuable tool to identify compounds responsible for reoccurring false positives that can inform better patient care and offer a more accurate picture of the continually evolving opioid epidemic.

B-309

Evaluation of CLIA Waived Point-of-Care Drug Screening Cups for Drugs of Abuse Testing in Ambulatory Clinics: Opportunities for Improvement

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Background: Kentucky is ranked 5th in the United States for opioid overdose deaths. Urine drug testing is recommended in addiction treatment programs to both assure compliance with prescribed medication and to monitoring treatment efficacy. Point-of-Care (POC) Urine Drug Screening (UDS) cups, one of the immunoassay-based UDS platforms, provides on-site qualitative drug screening that can simultaneously detect broad drug classes. The rapid preliminary results allow providers to adjust their monitoring or treatment plans during their office visit, significantly improving patient care and provider satisfaction. Ten University of Kentucky ambulatory clinics utilize CLIA waived POC drug test cups for pain management, compliance monitoring, and opioid addiction programs. In October 2019, with a sudden discontinuation of supply from the manufacturer, an alternative POC UDS screening test was required. **Objective:** To secure an alternative CLIA waived POC UDS cup with reliable performance and sustainable supply to our ambulatory clinics. **Methods:** Three CLIA waived POC UDS cups were evaluated: The ECO III, One Step Multi-Drug, and Preview Cups. All three UDS cups claimed simultaneous, qualitative detection of the following major drug classes: Methamphetamine, Amphetamine, Cocaine, Morphine, Oxycodone, Buprenorphine, Methadone, Marijuana, Benzodiazepines and Barbiturates. The efficacy of all three drug testing cups were evaluated using suitable urine specimens. For rarely encountered drugs negative urine specimen were spiked with corresponding reference standards. For common drugs positive patient specimens were utilized. All results were compared to the liquid chromatography- tandem mass spectrometry (LC-MS/MS) assay performed in the clinical toxicology laboratory at hospital. The positive and negative agreement was assessed by parallel comparison against the manufactures' claimed cut-offs. Precision was evaluated using quality control (QC) materials. The stability of test results was evaluated by checking the consistency of readings at time points of 5 min and 10 min post sample collection. **Results:** During the 3-month period study, about 100 urine samples were tested on each of the POC cups. The ECO III was eliminated due to inconvenient results display. The One Step Multi-Drug Cup and the Preview Cup both yielded a 100% positive agreement for all drugs except opiates (94%). Negative agreement was >97% and 85 - 100% for the One Step and Preview cups respectively. Both cups' results were stable up to 10 min post urine collection. The One Step Multi-Drug Cup was initially selected for its better overall agreement, but subsequently was problematic due to inconsistency of supplies and quality control failures on 30% of the cups. The Preview cup, displayed comparable performance, but consistent supply was unavailable. The inconsistency of the cups warranted a thorough educational program prior to roll out. **Conclusion:** In the ambulatory clinics, rapid and broad drug testing provided by POC urine drug screen cups plays an important role if applied appropriately. Evaluation of a reliable POC urine drug cup can be complexed by unexpected challenges. Drug cup users should be aware of many challenges in obtaining consistent supplies and cup reproducibility. Close oversight by ambulatory POC teams is necessary to ensure safe and effective results for urine drug cup testing.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Tumor Markers & Cancer Diagnostics

B-311

Clinical Significance of Circulating Tumor Cells in Ovarian Cancers

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Background: "Liquid biopsy", where body fluids is screened for biomarkers has attracted extensive attention. This study aims to examine the clinical significance of circulating tumor cells (CTCs) in epithelial ovarian cancers (EOC) in comparison to existing biomarkers cancer antigen 125 (CA125) and Human epididymis protein-4 (HE4).

Methods: Magnetic powder labeled monoclonal antibodies for epithelial cell adhesion molecule (EpCAM), Human epidermal growth factor receptor 2 (HER2), and Mucin 1 cell surface associated **(** MUC1) were prepared according to the manufacturer's protocol. Expression of six ovarian cancer related genes were detected in CTCs from EOC patients using multiplex reverse transcriptase-polymerase chain reaction (Multiplex RT-PCR). CTCs, CA125 and HE4 were measured in serial from 192 blood and 192 serum samples from 64 EOC patients and correlations with treatment were analyzed. CA125 and HE4 cutoffs were 35 kU/L and 140 pmol/L respectively. Immunohistochemistry was used to detect the expression of tumor-associated proteins in tumor tissues and compared with gene expression in CTCs from patients.

Results: CTCs were detected in 94% (60/64) of newly diagnosed patients. In newly diagnosed patients, the number of CTCs were correlated with stage (p=0.004). Patients with stage IA-IB disease had a CTC positive rate of 100% (11/11), much higher than the CA125 positive rates of 64% (7/11) and HE4 positive rates of 82%(9/11) for the same patients. The numbers of CTCs changed with treatment, and the expression of EpCAM (p=0.003) and HER2 (p=0.035) in CTCs was correlated with resistance to chemotherapy. Expression of EpCAM in CTCs before treatment was also correlated with overall survival (OS) (p=0.041).

Conclusion: Detection of CTCs allows early diagnosis in ovarian cancer patients. Expression of EpCAM in CTC positive patients predicts prognosis and should be helpful for monitoring treatment.

B-312

Right Place at the Right Time: Understanding the Importance of Temporal Expression of Biomarkers

C. N. Sharp, E. Stauble, S. Jortani. *University of Louisville, Louisville, KY*

Background: The concept of temporal changes in biomarkers through disease progression is not a new one and can be evidenced by BNP for heart failure, as well as albuminuria and eGFR for chronic kidney disease. In cancer research, biomarker research has gained popularity, especially with the development of large-scale proteomic and genomic approaches to discovery. Despite the ability to generate many novel biomarker candidates, 0-2 biomarkers are FDA approved per year across all diseases [1]. As a result, the "biomarker pipeline" has been closely compared to the drug discovery pipeline. As with drug discovery, the success of validation depends largely on the selection of the right population. Here, we present the importance of considering temporal expression of biomarkers as an additional dimension in the biomarker discovery and validation process. As a proof of concept, we will focus on Open Reading Frame 1 protein - (ORF1p), a product of epigenetic activity of Long-Interspersed Nuclear Element (LINE1), which has been indicated as a biomarker for many cancers.

Methods: Previously, we have developed an ELISA-based assay for detection of ORF1p in patient serum. Serum aliquots from patients at high risk of lung cancer development were collected with consent under IRB approval (AIRB- 16 July 2018). A total of 83 patients were included in this analysis. Serum aliquots from lung cancer patients within five years of diagnosis were collected under a separate, IRB-approved study (#13.0508). Eligible patients were diagnosed with non-small cell lung cancer within five years of enrollment. A total of 38 patients with varying stages of cancer were included for this analysis. Another cohort of well women and those diagnosed with endometrial cancer were enrolled with consent under IRB approval (16.0010). This cohort was recruited during routine, annual gynecological exams. In total, 20 cancer patients and 39 controls were included in our analysis. All samples were run using our ORF1p assay, and statistical analysis was performed using Prism 5.0 software.

Results: ORF1p levels were not statistically different in patients with endometrioid adenocarcinoma compared to those without cancer. This was also the case for our lung cancer (LC) cohort. However, ORF1p was elevated in the cohort of patients at high risk for lung cancer, as evidenced by the presence of lung nodules. In addition, ORF1p levels trended higher in low stage LC patients compared to those with advanced stage LC.

Conclusion: ORF1p highlights the importance of addressing temporal changes in biomarkers throughout the course of disease. Understanding the temporal expression of a biomarker may further aid in picking the right population of patients to validate the marker in. In turn, this could improve the number of biomarkers that make it through the discovery pipeline.

Reference:1. Paulovich AG, Whiteaker JR, Hoofnagle AN, Wang P. The interface between biomarker discovery and clinical validation: The tar pit of the protein biomarker pipeline. *Proteomics Clin Appl.* 2008;2:1386-1402.

B-313

Performance Evaluation of N Latex FLC kappa and Lambda Reagents on the Atellica CH Analyzer

D. Muth, M. Ehm, K. Schwarz. *Siemens Healthineers, Marburg, Germany*

Background:

The serum free light chain (FLC) immunoglobulins are essential biomarkers for clonal plasma cell disorders (PCD). The International Myeloma Working Group has provided consensus guidelines that aid in the diagnostics and management of these disorders. The adaptation of N Latex FLC kappa and N Latex FLC lambda reagents on the Atellica® CH Analyzer was investigated. Data for imprecision, method comparison, reference interval confirmation, and serum/plasma comparison were established.

Methods:

Latex-enhanced mouse monoclonal antibody reagents for FLC kappa and lambda were assayed on the Atellica CH Analyzer. CLSI guideline EP05-A3 was applied for the precision study to estimate repeatability and total CV. A method comparison study was conducted according to CLSI guideline EP09-A3. FLC kappa, FLC lambda results and the FLC kappa/lambda ratio were correlated with data generated on the BN ProSpec® System. CLSI guideline EP28-A3C was used for a conformity study of the U.S. specific reference intervals. A paired sample study was conducted according to CLSI guideline EP09-A3.

Results:

Repeatability for FLC kappa ranged from 0.67 to 1.16% and total CV from 1.14 to 2.04%, depending on the analyte concentration. For FLC lambda, repeatability ranged from 1.12 to 2.27% and total CV from 3.22 to 4.35%. Passing-Bablok regression results between the BN ProSpec System and Atellica CH Analyzer were $y = 1.00 x 0.074$ ($r^2 = 0.977$) for FLC kappa, $y = 0.925 x 0.061$ ($r^2 = 0.941$) for FLC lambda, and $y = 1.12 x -0.032$ ($r^2 = 0.995$) for the FLC ratio. Reference interval confirmation revealed within-range recoveries of 96.0% for FLC kappa, 95.3% for FLC lambda, and 97.3% for the k/l ratio. Passing-Bablok regression results between serum and EDTA plasma were $y = 1.01 x -0.388$ ($r = 0.997$) for FLC kappa and $y = 1.07 x -1.056$ ($r = 0.984$) for FLC lambda.

Conclusion:

N Latex FLC kappa and N Latex FLC lambda reagents were applied successfully on the Atellica CH Analyzer. The FLC applications showed good correlation with the commercialized immunoassays on the BN ProSpec System. Excellent precision was proven. Capability for use with either serum or EDTA plasma was confirmed. Overall, the FLC applications on the Atellica CH Analyzer are attractive for clinical laboratories with high sample throughput.

Disclaimer: Product availability may vary from country to country and is subject to varying regulatory requirements.

B-316

Chromosomal Abnormalities Detected by Multiprobe Fluorescence In Situ Hybridization (FISH) in Brazilian Chronic Lymphocytic Leukemia Patients

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Background: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia. Cytogenetic abnormalities play a major role in the prognosis of patients with CLL. Prognosis is related to clinical staging and cytogenetic findings. The most common abnormalities include 11q deletions, trisomy 12, 17p deletions and 13q deletions. Patients with a 13q deletion as the sole abnormality have the best prognosis. The worst prognosis has been observed in patients with 17p deletion (or p53 mutation). Detection of chromosome abnormalities by conventional metaphase cytogenetics has been challenging due to limited proliferation of CLL cells in vitro. Interphase fluorescence in situ hybridization (I-FISH) using probes specific for the most common abnormalities is generally considered the gold standard approach. To investigate the frequency of chromosomal abnormalities in clinical samples from CLL patients in a Brazilian private laboratory. **Methods:**In order to analyze FISH results of Brazilian patients, we conducted a retrospective case study in CLL patients at the Hermes Pardini Institute database between 2014 and 2019. The FISH reaction was performed using a panel of probes for chromosomes: 6q23.3 (MYB), 11q22 (ATM), 12, 13q, 14q32 (IGH), t(14;18) (IGH/BCL2), t(11; 14) (IGH/CCDN1) and 17p (p53). **Results:** The present retrospective study included 155 patients: 97 male and 58 female patients. The median age of patients was 61 years (range: 32-86). Chromosomal abnormalities were found in 61.5% of patients and presented as follows: single 13q14.3 deletion (64.6%), trisomy 12 (31.3%), 17p13.1 deletion (15.6%), t(11;14) (CCND1/IGH) translocation (14.6%). Forty patients (41.7%) presented two or more chromosomal abnormalities simultaneously. Considering del(13q), 62 patients presented this deletion, with 59 (95.2%) being monoallelic and three (4.8%) both monoallelic and biallelic (mosaic). Del(13q) associated with trisomy 12 was detected in thirteen cases. Del(17p) was not detected as the sole abnormality in any of our patients. Considering del(11p), three (27.3%) patients presented this deletion as the sole aberration. The median age of the three patients with isolated del(11q) was 49.7 (range: 42-56) years. **Conclusion:** The application of I-FISH) to CLL has substantially enhanced our ability to detect chromosomal abnormalities. In most papers only del(13q), del(17p), del(11q) and trisomy 12 are investigated by FISH. However, it is known that other aberrations can be correlated to CLLand they may influence the prognosis and response to treatment. In this study, additional aberrations were investigated using eight probes. Our results did not significantly differ those other studies.

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Serum Exosomal miR-1290 as a Potential Diagnosticand and Prognostic Biomarker of Lung Adenocarcinoma

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Background Lung cancer is the leading cause of cancer-related death worldwide. The most common histological subtype is lung adenocarcinoma (LUAD). Currently, the majority of lung cancers are diagnosed at advanced stages with an overall five-year survival rate less than 15%. Extracellular miRNAs have attracted major interest as a method to diagnose tumorigenesis or evaluate prognosis. Exosomal miRNAs, composed of cell-free nucleic acids, could be novel and functional biomarkers for cancer detection. The aim of this study was to identify specific miRNAs in exosome as serum biomarkers for the detection and prognosis of LUAD. **Methods** We used a first set of 20 serum samples as a screening cohort, including 10 LUAD patients and 10 healthy volunteers matched for age and sex as negative controls. Subsequently, selected microRNAs were evaluated on an independent larger group of samples (90 specimens: 30 early LUAD, 30 advanced LUAD and 30 healthy controls). Serum exosomes extracted were verified using transmission electron microscopy, nanoparticle tracking analysis and western blot. The concentrations of miRNAs were measured through qRT-PCR. Candidate miR-1290 was identified by literature retrieval and clinical samples. ROC and survival analysis were used to evaluate the diagnosis efficiency and prognosis value of exosomal miR-1290 in LUAD. **Results** Serum exosomal miR-1290 was highly expressed in LUAD [healthy control: 689.84 (419.95, 915.17) fmol/l; early LUAD: 1696.95 (1032.26, 2461.99) fmol/l; advanced LUAD: 4547.65 (2987.38, 7060.30) fmol/l; P<0.001] and dramatically decreased after resection (P=0.003). Its concentration was correlated with gender (P=0.038), tumor stage (P<0.001), tumor size (P<0.001), lymph node (P=0.003) and distant metastasis (P<0.001), smoking (P=0.015) and alcohol consumption history (P=0.005). Compared with traditional tumor markers, serum exosomal miR-1290 had a higher diagnostic efficacy of LUAD (AUC: 0.937, P<0.001), with a sensitivity of 80% and a specificity of 96.7%. Kaplan-Meier survival analysis showed that progression-free survival rate of patients with higher serum exosomal miR-1290 was significantly lower than the low expression group (Log-rank P<0.001). Cox proportional hazards model analysis showed that exosomal miR-1290 could be an independent risk factor for the prognosis of LUAD (HR=7.80, P=0.017).**Conclusions** Serum exosomal miR-1290 may be a potential biomarker for the diagnosis and prognosis of LUAD.

B-319

A Dual Marker Methylation Classifier for Non Invasive Early Detection of Bladder Cancer

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Background Bladder cancer (BC) is a common lethal urinary tract malignancy with high recurrent rate. The survivals and prognosis can be significantly improved by early detection and monitoring. However, the gold standard of BC diagnosis, cystoscopy, is invasive and causes great discomfort to patients while non-invasive approaches, including urinary test of fluorescence in situ hybridization (FISH), lack satisfactory sensitivity especially for early diagnosis. We aimed to develop a urine-based DNA methylation assay for BC early detection. **Methods** The study analyzed DNA co-methylation profiles of 22 genomic regions in 204 urine samples (105 BC and 99 non-BC cases) using a multiplex quantitative PCR (qPCR) assay. By comparing methylation levels of each region between BC and non-BC groups, the best performing markers were identified to construct a binary classifier. Clinical performance of the classifier was further validated in an independent retrospective cohort consisting of 107 participants (72 BC and 35 non-BC samples). **Results** Among 22 genomic regions, methylation levels of ONECUT2 and VIM were found to be significantly different (p<0.0001) between BC and non-BC populations. A classifier based on ONECUT2 and VIM methylation levels further revealed a sensitivity of 85.7% (95% CI:79.0%–92.4%) and specificity of 82.8% (95%CI: 75.8%–89.9%) for BC detection. The dual-marker classifier showed consistent performance characteristics in the independent validation cohort with an AUC of 0.89, sensitivity of 87.5% (95% CI:77.6%–94.1%) and specificity of 91.4% (95% CI:76.9%–98.2%). Compared to the performance of FISH within the cohort, the classifier achieved superior sensitivity of 83.3% (25.0% by FISH) and 93.8% (65.6% by FISH) for detection low-grade BC and non-muscle invasive bladder cancer (NMIBC), respectively. **Conclusions** Dual-marker methylation classifier of ONECUT2 and VIM features high BC detection sensitivities particularly for low-grade tumor and NMIBC, providing a sensitive and accurate BC early diagnostic tool as alternatives of current non-invasive assays.

Detection sensitivities (%) in validation cohort	Low-grade BC	High-grade BC	NMIBC	MIBC
Dual-marker methylation classifier	83.3%	88.5%	93.8%	80.0%
FISH	25.0%	78.8%	65.6%	76.7%

B-323

Investigation of Nonlinear Specimens in Two Chromogranin A Assays

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Background: Chromogranin A (CgA) is a prohormone found in the secretory granules of neuroendocrine cells. The protein contains several cleavage sites, resulting in several functional peptides including pancreastatin, catestatin, prochromacin and vasostatin I and II. Additionally, serum CgA concentrations have shown clinical utility for the detection and monitoring of functional and non-functional neuroendocrine tumors.

Previous studies at our facility identified a CgA ELISA (Chromogranin A ELISA kit, Cisbio Bioassays) absent of an apparent high-dose-hook effect (nonlinearity upon dilution) that was observed at a rate of approximately 15% of elevated specimens in three other CgA immunoassays. (Erickson et al, 2016). However, after routine testing

of tens of thousands of specimens at neat and 5-fold dilution over the past few years, it was observed that a very small percentage (approximately 0.03%) of specimens do exhibit nonlinearity when tested using this ELISA. An evaluation of the newer analyzer based CgA assay (B·R·A·H·M·S CgA II KRYPTOR, Thermo Fisher Scientific, Inc.) produced similar results.

The aim of our study was to investigate these unique nonlinear specimens in an attempt to uncover any clues as to why they exhibit this phenomenon. Particularly, using CgA assays that have demonstrated a high resilience to hooking and nonlinearity as compared to other CgA assays. Possible causes considered were heterophilic antibody (HAMA) interference, macromolecule effects and elevated serum creatinine due to probable kidney failure.

Methods: Serum CgA was measured according to each assay manufacturer's testing protocol. Highly elevated CgA serum specimens showing good linearity upon dilution (n = 50), and 20 specimens demonstrating nonlinearity using the Cisbio ELISA, were tested with the KRYPTOR CgA II assay neat and at multiple dilutions. HAMA blocking tubes and protocols were used for HAMA interference studies and polyethylene glycol (PEG) precipitation methods for macromolecule investigations. Serum creatinine was measured using a Roche cobas c702 (Roche Diagnostics Corp.).

Results: Of the 50 highly elevated specimens demonstrating good linearity from Cisbio CgA testing, 49 also showed good linearity using the KRYPTOR CgA assay. All 20 specimens exhibiting nonlinearity using the Cisbio ELISA were also nonlinear to varying degrees utilizing the KRYPTOR assay. Specimens demonstrating strong nonlinearity (n = 6), were retested for HAMA interference using the KRYTOR assay, generating differences (HAMA block - no HAMA block) ranging -3.2 – 4.2%. CgA recoveries from PEG precipitation studies ranged 157 – >5714% for affected specimens vs. 71 – 79% for normal specimens. Eight of 14 nonlinear specimens (57%) produced elevated serum creatinine results (>1.20 mg/dL), with three of those considered as extremely elevated (5.13 – 9.19 mg/dL).

Conclusions: Long term observation of CgA testing using the Cisbio CgA ELISA indicates that a small number (0.03%) of specimens demonstrate nonlinearity upon dilution. A limited evaluation of the B·R·A·H·M·S CgA II KRYPTOR assay suggests similar performance in terms of nonlinearity when testing elevated CgA specimens. Although efforts to identify a cause of this effect in these unique specimens were inconclusive, results do suggest that neither HAMA interference, macromolecule effects nor renal failure are major factors causing the phenomenon.

B-324

Tumor Specific Protein 70 Contributes to Immune Escape in Non-Small Cell Lung Cancer

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Background: A novel tumor marker tumor specific protein 70 (TSP70 or SP70) was previously demonstrated to promote the proliferation, invasion and metastasis of non-small cell lung cancer (NSCLC). However, it is unclear whether SP70 can mediate tumor immune escape. This study was to investigate the relationship between SP70 and the proportion of immunosuppressive cells such as $CD4^+$Tregs and $CD8^+$Tregs in NSCLC patients. Since $CD4^+$Treg cells account for a higher proportion of T cells, the effect of SP70 on the differentiation of these cells was first explored.

Methods: Serum and peripheral blood of 37 newly diagnosed NSCLC patients and 26 healthy subjects from the First Affiliated Hospital of Nanjing Medical University were collected. Serum SP70 concentration was detected by ELISA, and CEA, NSE and CYFRA21-1 concentrations were determined by electrochemical luminescence. Peripheral blood mononuclear cells (PBMCs) were isolated and the proportions of $CD4^+CD25^+Foxp3^+$Tregs and $CD8^+CD28^-Foxp3^+$Tregs were detected by flow cytometry. Spearman correlation analysis was used to investigate the correlation between SP70 and $CD4^+/CD8^+$Tregs. In addition, purified SP70 protein, SPC-A1 supernatant with high concentration of SP70 and SPC-A1 supernatant with low concentration of SP70 (SP70 was removed by affinity chromatography column) were used to culture PBMCs derived from 11 healthy donors for 5 days in vitro to determine the effect of SP70 on the differentiation of $CD4^+$Tregs.

Results: Total of 37 newly diagnosed NSCLC patients and 26 healthy controls (HC) were enrolled. The level of serum SP70 and the proportions of $CD4^+/CD8^+$Tregs in PBMCs were significantly higher in NSCLC group than in HC group. Moreover, the proportions of $CD4^+/CD8^+$Tregs were significantly higher in NSCLC patients with serum SP70>7.5 ng/ml than in those with SP70≤7.5 ng/ml ($P<0.05$, $P<0.01$). Spearman correlation analysis revealed that only SP70 rather than other tumor marker including CEA, NSE and CYFRA21-1 was significantly associated with the proportions of $CD4^+$ ($P<0.05$, Spearman r=0.4276) or $CD8^+$Tregs ($P<0.05$, Spearman r=0.4006). Besides, Patients with advanced stage and lymph node metastasis had significantly higher SP70 levels and proportions of $CD4^+/CD8^+$Tregs. *In vitro* results showed that the proportion of $CD4^+$Tregs in the treatment group with purified SP70 protein was significantly higher than that in the blank control group ($P<0.05$), while in the SPC-A1 supernatant culture groups, the proportion of $CD4^+$Tregs decreased obviously after the removal of SP70 ($P<0.05$).

Conclusion: SP70 plays a key role in inducing Treg cell differentiation and could potentially mediate tumor immune escape in NSCLC.

B-325

Early Detection of Tumor Specific Protein 70 Positive Circulating Tumor Cells in Non-Small Cell Lung Cancer

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Background: SP70 is a novel tumor marker for non-small cell lung cancer (NSCLC) in recent years. However, the application of SP70 as a marker of specific circulating lung cancer cells (SCLCCs) and serum in the early diagnosis of NSCLC has not been investigated.

Methods: In this prospective study, a total of 139 subjects from the First Affiliated Hospital of Nanjing Medical University were enrolled, including 72 patients with NSCLC who underwent chemotherapy or surgery as a treatment regimen, 19 patients with benign pulmonary disease and 48 healthy controls. Peripheral blood circulating tumors cells (CTCs), namely SCLCC, serum SP70, carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA21-1) levels were detected. ROC curve was made to determine the diagnostic performance of SCLCC and serum SP70 in NSCLC.

Results: The positive rate of SCLCC of NSCLC patients was 62.50%, significantly higher than that of serum CEA and CYFRA21-1 (41.67% and 43.06%). SCLCC and SP70 levels in NSCLC patients were significantly higher than those of non-NSCLC (including benign lung diseases and healthy controls) cases ($P<0.001$ for SCLCC and $P<0.001$ for SP70, respectively). Analysis of ROC demonstrated that the combination of SCLCC and serum SP70 could improve the diagnostic performance of NSCLC, as well as in early-stage NSCLC (AUC=0.889). Furthermore, the combination showed superiority in detecting early-stage NSCLC (sensitivity 87.18%, specificity 82.09%), and they maintained predominance in distinguishing CEA & CYFRA21-1-negative NSCLC patients with the AUC was 0.895 (sensitivity 87.10%, specificity 82.09%). Meanwhile, SCLCC and SP70 levels dramatically decreased after chemotherapy ($P<0.001$ for SCLCC and $P<0.001$ for SP70, respectively) or surgery ($P=0.004$ for SCLCC and $P<0.001$ for SP70, respectively), while the levels of CEA and CYFRA21-1 were not changed obviously.

Conclusion: These findings highlight that peripheral blood SCLCC and serum SP70 are sensitive indicators of early diagnosis of NSCLC, and can be used to evaluate the therapeutic efficacy of NSCLC patients underwent chemotherapy or surgery.

B-326

SP70 Targeted Tumor Cell Enrichment and Analysis in Body Fluids

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Background: Currently, the diagnosis of malignancy in body fluids, including the detection of circulating tumor cells (CTCs) in blood, suffer low sensitivity due to the lack of specific target for tumor cell enrichment and identification. In the previous study, we produced a monoclonal antibody designated NJ001, which targets tumor specific antigen SP70. The aim of this study is to assess the value of NJ001 coated magnetic beads capturing technique in distinguishing benign and malignant body fluids samples.

Methods: In the present study, 261 body fluids specimens were obtained and analyzed from Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University (NMU) and Department of Pathology and Laboratory Medicine, University of California, Los Angeles (UCLA), between March and July 2017. Tumor cells were enriched by the NJ001 monoclonal antibody coated magnetic beads. SP70 positive cells were detected by microscopic examination after Papanicolaou staining. CTCs from 13 patients were sequenced by Next Generation Sequencing (NGS).

Results: Comparing with routine cytology technique, cytology with SP70 targeted immuno-magnetic beads increased the sensitivity and accuracy. The accuracy of with SP70 targeted cytology and routine cytology was 82.4% and 55.6%, respectively. In

a follow-up study, 68 of 76 patients, who were previously diagnosed benign disease with negative cytology result but positive in our new technology with SP70, were confirmed malignant by MRI, needle biopsy and NGS.

Conclusion: Our results showed that SP70 could be a novel biomarker for identifying and distinguishing benign and malignant body fluids samples and enrich tumor cells for subsequent molecular analysis.

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Clinical Value of Tumor Specific Protein 70 in the Diagnosis and Prognosis Prediction of Hepatocellular Carcinoma

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Background: Tumor specific protein 70 (TSP70 or SP70) was identified as a new biomarker associated with proliferation and invasion of cancer cells. This study aims to assess the clinical value of SP70 in the diagnosis and prognosis prediction especially early recurrence of hepatocellular carcinoma (HCC).

Methods: The expression of SP70 in tissues from liver diseases and cell lines was determined by immunohistochemistry, immunofluorescence, flow cytometry and western blot. In this cross-sectional study, a total of 552 subjects from the First Affiliated Hospital of Nanjing Medical University were recruited, including 183 HCC patients, 155 patients with benign liver diseases (BLD), 27 patients with intrahepatic cholangiocarcinoma (ICC) and 187 healthy individuals. Serum levels of SP70, alpha-fetoprotein (AFP) and prothrombin induced by vitamin K absence II (PIVKA-II) were detected, and the diagnostic performance of single and combined tests for HCC were obtained using receiver operating characteristic (ROC) curves. Univariate and multivariate analysis were performed to identify factors predictive of recurrence-free survival (RFS).

Results: SP70 was highly expressed in hepatocellular carcinoma tissues, while other liver cancer types and paracancer tissues showed a low expression pattern. Besides, SP70 was highly expressed in HCC cell line HpeG2, and mainly expressed on cell membrane and in cytoplasm. Serum SP70 levels in HCC group were significantly higher than those in ICC group, BLD group and healthy control (HC) group. ROC analysis showed that areas under the curve (AUC) of SP70 for detecting HCC (AUC=0.856) was second only to PIVKA-II (AUC=0.857). While the combined detection of SP70 and AFP showed the best diagnostic performance (AUC=0.918) in all three combinations. The serum SP70 levels after resection in the recurrence group was significantly higher than that before resection (P<0.001). Univariate and multivariate analysis confirmed SP70 and microvascular invasion (MVI) as highly significant predictors of early recurrence. Focused on individual biomarkers, only SP70 was independently associated with RFS. The median RFS of patients with higher SP70 levels was shorter than that of patients with lower SP70 levels (6.0 months vs 9.0 months, P<0.001).

Conclusion: SP70 can be used as a valuable marker in the diagnosis of HCC when combined with AFP. What's more, SP70 is a sensitive indicator in detecting early recurrence of HCC after resection compared to AFP and PIVKA-II.

B-328

Detection of miRNAs in Prostate Tissue, Plasma and Urine of Prostate Cancer Brazilian Patients

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Background: Prostate cancer (PCa) is the second most prevalent oncologic disease among men worldwide. The main parameters for PCa diagnosis are prostate-specific antigen (PSA) test and digital rectal examination (DRE). However, these diagnostic tools are relatively limited (low specificity and sensitivity). Expression of microRNAs (miRNAs) is markedly deregulated in many types of cancers, and can be seen as potential candidates for PCa detection. The aim of this study was identifying PCa-specific expression profile of miRNAs in prostate tissue, as well circulating miRNAs from blood and urine as noninvasive molecules for diagnosis. **Methods:** Using TaqMan Custom Array MicroRNA cards by RT-qPCR, we measure the concentration of 44 preselected miRNAs in prostate tissue, plasma and urine samples from a case-control Brazilian population, including 22 PCa patients diagnosed by prostate biopsy (cases) and 28 patients (controls), whose biopsy samples contained only normal prostate cells and benign glands. **Results:** Of these 44 miRNAs, 36 (81.8%) had detectable expression in prostate tissue in more than 30% of the samples. Six of these 36 miRNAs (miR-148a-3p, miR-18a-5p, miR-200b-3p, miR-205-5p, miR-21-5p and miR-375) were differentially expressed between cases and controls (p<0,05). For plasma and urine samples, 21 (47.7%) and 19 (43.2%) miRNAs had detectable expression, but only 5 (miR-100-5p, miR-200b-3p, miR-214-3p, miR-375 and miR-548am-5p) and 2 miRNAs (miR-21-5p and miR-375) were significantly expressed (p<0,05), respectively. All miRNAs differentially expressed in these three specimens were upregulated in PCa group compared with controls, except to miR-205-5p that was downregulated in the prostate tissue samples of the PCa group. MiR-375 was upregulated in PCa patients in prostate tissue, plasma and urine, becoming a potential biomarker to be evaluated. **Conclusion:** We successfully identified a miR-375 signature that could predict the presence of PCa from plasma and urine samples. Prospective validation is needed for PCa-specific miRNAs could assist in more specific detection of PCa.

B-329

Analytical Performance of Automated Thrombospondin-1 and Cathepsin D Immunoassays for Prostate Cancer Risk Assessment

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Background: PSA-based prostate cancer testing is under debate in the medical community as it leads to overdiagnosis, negative biopsies and overtreatment of patients. Proclarix® is a novel diagnostic test that acts as a decision aid for patients with unclear PSA results. A risk calculator combines the test results of the serum-markers total PSA, free PSA, thrombospondin-1 (THBS1) and cathepsin D (CTSD) with the patient's age to calculate a prostate cancer risk score. As automation is a vital part of clinical laboratories to manage testing workload and maximize efficiency, we evaluated precision and recovery of the THBS1 and CTSD immunoassays on the automated Dynex DSX® immunoassay analyzer. **Methods:** THBS1 and CTSD immunoassays (Proteomedix, Zurich, Switzerland) were implemented at the ZLM (Center for Laboratory Medicine, St. Gallen, Switzerland) on existing DSX instruments (Dynex Technologies Inc, Chantilly, VA, USA) using assay files provided by the manufacturer. Automated measurements of 16 serum samples were performed over six days and compared to manual measurements of the same samples over six days. Recoveries were calculated using the median concentrations of the manual measurements as references. Within-run, between-run and total precision were calculated according to CLSI guideline EP5-A2. **Results:** Both assays showed within-run (THBS1: 3.5%, CTSD: 3.9%), between-run (THBS1: 3.0%, CTSD: 1.5%) and total precision (THBS1: 4.7%, CTSD: 4.2%) coefficients of variation (CV) below 5%. Recoveries for all measurements are shown in Figure 1. The mean biases of automated compared to manual measurements were -4.0% for THBS1 and -1.8% for CTSD. **Conclusion:** Automated processing of the THBS1 and CTSD immunoassays suits the requirements posed by the workflow of clinical laboratories and increases workflow efficiency. Concentrations determined on the Dynex DSX were comparable to values obtained by manual processing of the immunoassays and the precision was similar.

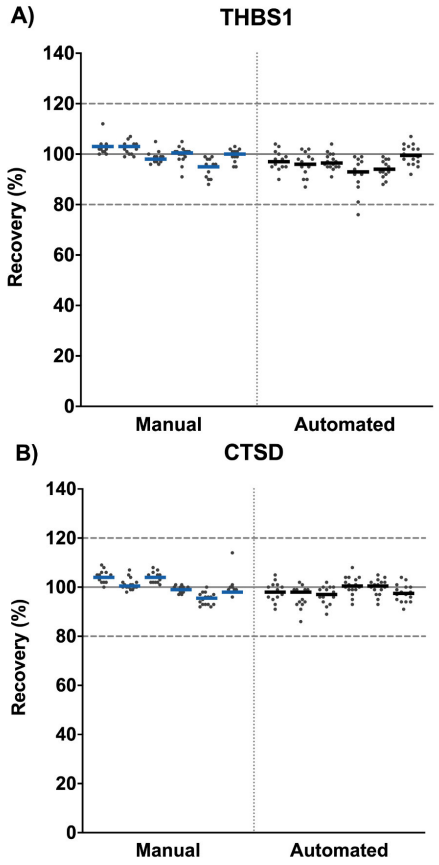

Figure 1: Recoveries of 16 samples measured six times manually and six times on an automated ELISA analyzer. A) THBS1 and B) CTSD. 100% = median concentrations of the manual measurements.

B-330

Analytical Validation of the Proclarix Risk Score for the Diagnosis of Prostate Cancer

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Background: Controversy continues to roil around the role of PSA testing for prostate cancer detection. Elevated PSA can be caused by multiple factors other than cancer leading to unnecessary biopsies, overtreatment, patient anxiety and unnecessary healthcare spending. Better tools to select patients for prostate biopsy, particularly in the diagnostic grayzone between 2 and 10 ng/ml PSA, are needed. Proclarix® is a novel multianalyte assay with algorithmic analysis (MAAA) that combines serum concentrations of thrombospondin-1 (THBS1), cathepsin D (CTSD), total PSA (tPSA), free PSA (fPSA), and patient age to calculate a risk score for clinically significant prostate cancer. The objective of this study was to evaluate the feasibility of implementing Proclarix in the routine workflow of a clinical laboratory. **Methods:** Proclarix (Proteomedix, Zurich, Switzerland) was established and conducted at the Center for Laboratory Medicine (St. Gallen, Switzerland) according to manufacturer instructions. tPSA and fPSA were measured on a UniCel® DxI800 instrument using Access® Hybritech reagents (Beckman Coulter Inc, USA). THBS1 and CTSD were measured on a DSX® workstation (Dynex Technologies, USA) using Proclarix reagents. The Proclarix Risk Calculator software (Proteomedix, Zurich, Switzerland) was used to calculate risk scores. Reproducibilities were determined by measuring eight serum samples over six days. **Results:** A total of 48 risk scores were generated based on 192 analyte measurements. Risk scores of all measurements and coefficients of variation (CV) per sample are shown in Figure 1A. Mean total CV were 2.7%, 5.6%, 3.1%, 5.9% and 5.0% for tPSA, fPSA, THBS1, CTSD and the risk score respectively. Linear regression analysis between manufacturer assigned risk scores and measured average risk scores showed high linear relationship (Figure1B). **Conclusion:** Precision of the risk score was high and the risk score correlated well with target values. The implementation of Proclarix in the clinical laboratory workflow was efficient and successful.

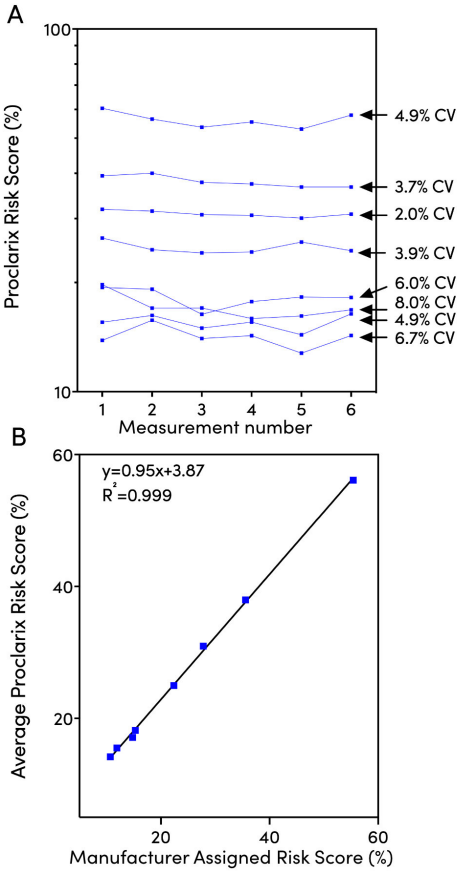

Figure 1: Proclarix Risk Score. (A) Eight samples measured over six days. CV's of samples are indicated on the right. (B) Linear regression analysis between manufacturer assigned risk scores and average risk scores measured at Center for Laboratory Medicine.

B-331

Serum Based Multiplex Protein Assay for Early Detection of Colorectal Cancer and Precancerous Lesions in a FIT Positive Population

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Background: Colorectal cancer (CRC) is the second leading cancer worldwide in terms of incidence, 5-year prevalence and mortality for both women and men ages 45 years old and up. The current screening method for many countries with organized screening programs is the FIT test for fecal occult blood; however, this test can result in false positive rates as high as 65%. A FIT reflex test could reduce unnecessary colonoscopies while reducing wait times for those patients that need confirmatory colonoscopies the most.

Methods: Danish FIT positive colonoscopy confirmed serum samples (n = 1,499) were divided into training and validation sets maintaining approximately equivalent percentages of clean colonoscopy (40%), low risk adenomas (16%), medium risk adenomas (19%), high risk adenomas (13%), stage I CRC (5%), stage II CRC (2%), stage III CRC (4%), and stage IV CRC (0.5%). Proteins were quantified by custom 16-plex immunoassays utilizing the Luminex xMAP® platform. A support vector machine supervised machine learning algorithm was trained with the 16 biomarkers plus age

and FIT concentration using 1,291 samples for the outcome medium risk adenoma, high risk adenoma, and CRC. Then this algorithm was tested on a blind 208 sample validation set.

Results: The training set was 90% sensitive and 27% specific (AUC = 0.68) and the validation set was 93% sensitive and 21% specific (AUC = 0.63). The sensitivities of the validation by risk/stage was as follows: medium risk adenoma 91%, high risk adenomas 92%, stage I CRC 100%, stage II CRC 100%, stage III CRC 100%, stage IV CRC 93%.

Conclusions: This study demonstrates feasibility of a novel blood-based multiplex protein immunoassay for use as a reflex to FIT positive results in population wide screening. It detected nearly all adenomas and carcinomas while reducing FIT false positives and thus unnecessary colonoscopies by more than 20%. A FIT reflex test could alleviate endoscopy burden experienced in countries with organized cancer screening programs, while providing better patient outcomes by detecting polyps and early-stage CRC with high sensitivity.

B-332

Use of Real Time Surveillance and QC Software Data to Support Extension of MAS T-Marker Control Product

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Background: The core laboratory is facing challenges to increase efficiency in its delivery of consistent results. Independent controls, provided by third party manufacturers, are routinely used by the core laboratory for monitoring their quality to ensure accurate results are being reported. Extended stability of controls enables users to utilize one lot of control for a longer period of time. This improves lab efficiency and productivity because less time and reagents are spent switching from one lot of control to another. Thermo Scientific™ MAS™ Tumor T-Marker™ Control Products have shelf life claim of 36 months. With the real time stability data collected, the shelf life of these products may extend to 60 months. Objective: The objective of this study was to assess the shelf-life stability of MAS Tumor T-Marker multi-constituent independent control, beyond the current three-year product shelf-life claim. Methods: Real time testing data from Thermo Fisher Scientific surveillance program along with customer reported data generated by Thermo Scientific™ LabLink xL™ QC software were utilized during the study. Real time stability data as well as in-use customer data were assessed for multiple lots of control material up to a period of five years. Data for 18 analytes on various testing platforms were collected and analyzed. The acceptance criteria is +/- 20%. Results: It was found that 16 analytes demonstrated consistent stability profiles over a 5 year period. The top performing analyte was AFP, which showed less than 3.5% drift over the period of the study. This was evident across 2 instrument models, including Beckman™ Access™ and Siemens™ Advia™ analyzers. Conclusion: The data from this stability study suggests that the analytes in the MAS Tumor T-Marker control may remain stable for longer than the current shelf life claim. The analytes in monitoring demonstrated stability for 5 years. Overall, increased stability benefits laboratories by reducing lot changeover, which increases consistency of analytical quality monitoring and increases laboratory efficiency, capability and productivity.

B-333

Alinity i ROMA Assay: A Precise Fully Automated Method for the Assessment of Ovarian Adnexal Mass

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Background: Alinity i ROMA (Risk of Ovarian Malignancy Algorithm) is a qualitative serum test that combines the results of Alinity i HE4, Alinity i CA125 II, and menopausal status into a numerical score. The ROMA assay is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery.

Methods: The Alinity i HE4 assay is a two-step immunoassay for the quantitative determination of HE4 antigen in human serum using chemiluminescent microparticle immunoassay (CMIA) technology. The Alinity i CA125 II assay is a two-step immunoassay for the quantitative determination of OC 125 defined antigen in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. A precision study was conducted by assaying 5 sera expressing varying amounts of CA125 and HE4 to simulate the ROMA score range for both premenopausal and postmenopausal subject categories in replicates of 2 at two separate times of the day for 20 days on the Alinity i system.

Results: Acceptance criterion for suitable performance was based on the Predictive Index (PI), a logit used to calculate the ROMA score by inverse logistic transformation, and whose measurement standard deviation should be close to constant over the whole range of the ROMA assay. The criterion for the precision study was that the PI value has a standard deviation of no more than 0.24 and 0.13, for a premenopausal patient and postmenopausal, respectively. Results demonstrated a maximum total SD of 0.090 and corresponding maximum total %CV ≤ 8.5% for the premenopausal subject category. A maximum total SD of 0.045 and corresponding maximum total %CV ≤ 4.2% was demonstrated for the postmenopausal subject category.

Conclusion: The Alinity i ROMA assay has demonstrated to be precise in the determination of the ROMA score using both the premenopausal and postmenopausal algorithms.

B-334

Cytogenetic Changes in Brazilian Patients with a Diagnostic Hypothesis in Myelodysplastic Syndrome (MDS)

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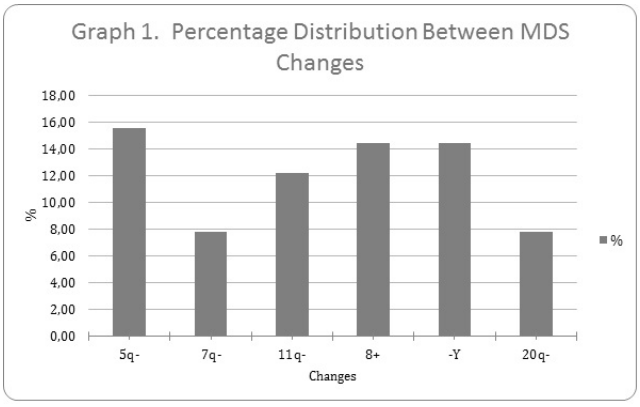
Background: A Myelodysplastic Syndrome (MDS) represents an heterogeneous group of bone marrow clonal defects characterized by the presence of displaced maturation of philatelic hematopoietic cells to one or more peripheral cytopenias and is prone to progress to chronic leukemia. In most cases, it presents cytogenetic anomalies in more than 50% of cases.

Objectives: Identify the frequency of cytogenetic changes in patients with a diagnostic hypothesis of MDS.

Methods: Using a statistical survey of men and women who underwent a karyotype exam in 2019 at DASA's cytogenetics laboratory in São Paulo, Brazil.

Results: In 2019, 621 samples of patients with a diagnostic hypothesis of MDS were sent, among these cases 90 (14.5%) karyotypes were altered. 37 female patients (mean age 70 years) and 53 males (mean age 75 years). The alterations with the highest frequency index were the deletions: 5q- (15.56%), 11q- (12.22%), 20q- (7.78%) and 7q- (7.78%), an nulisomy Y (14.44%) and trisomy 8 (14.44%) as shown in Graph 1.

Conclusion: Myelodysplastic Syndrome predominantly occurs in elderly males. The study showed that the cytogenetic evaluation of bone marrow in patients with MDS is of fundamental importance, as it not only confirms the diagnosis but is of great value in assessing prognosis, evolutionary monitoring and therapeutic choice.



B-335

The Lumipulse® G1200 CA19-9-N Assay: A Fully Automated Method for Quantitative Determination of CA 19-9 in Human Serum and Plasma

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Background: Carbohydrate antigen 19-9 (CA 19-9) is a sialylated Lewis A blood group antigen with a molecular weight of 10 kilodaltons. CA 19-9 is highly rated as an indicator for supplementary diagnosis and therapy evaluation in colorectal, pancreas, bile duct and gall bladder cancers. This study was conducted to analytically verify the Lumipulse G CA19-9-N assay and clinically validate the assay for monitoring recurrence and progression of cancer of the exocrine pancreas.

Methods: Lumipulse G CA19-9-N assay is a Chemiluminescent Enzyme Immunoassay for the quantitative determination of CA 19-9 in human serum and plasma on the Lumipulse G1200 System via a two-step sandwich immunoassay method using two monoclonal antibodies against CA 19-9. The amount of CA 19-9 in the specimen is obtained from the luminescence signals derived from the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt).

Results: The Lumipulse G CA19-9-N assay demonstrated linearity from 0.7 U/mL to 531.3 U/mL, and an analytical sensitivity with Functional Sensitivity ≤ 0.57 U/mL. A precision study of 6 sera (n = 80 for each sample) assayed in replicates of 2 at two separate times of the day for 20 days revealed a total %CV ≤ 5.3%. There was no High Dose Hook effect with up to 200,000 U/mL of CA 19-9 antigen in samples. Interference studies showed an average percent difference ± 10% between test and control samples for potential interferents, including 9 endogenous substances (human anti-mouse antibody, rheumatoid factor, conjugated bilirubin, unconjugated bilirubin, human immunoglobulin G, biotin, triglycerides, hemoglobin, and human serum albumin) and 12 drugs (5-Fluorouracil, Cisplatin, Cyclophosphamide, Cytarabine, Doxorubicin HCl, Gemcitabine, Leucovorin, Methotrexate, Paclitaxel, Pegylated Liposomal Doxorubicin (Doxil®), Streptozotocin, and Tamoxifen), which were spiked individually into sera (test samples). A comparison of Lumipulse G CA19-9-N with the predicate, ARCHITECT CA19-9XR, was analyzed using weighted Deming regression. The slope and correlation coefficient (r) obtained were 1.3179 and 0.6628, respectively, for the tested specimens (n = 84). An extended analysis was performed on specimens measuring above the range of both devices (n=103). The slope and correlation coefficient (r) obtained were 1.3198 and 0.7380, respectively. In a monitoring study, changes in CA 19-9 levels in serial serum samples from 83 subjects diagnosed with cancer of the exocrine pancreas were compared to changes in disease status. A total of 374 pairs of observations were recorded with an average number of 5.6 observations per patient. A positive change in CA 19-9 was defined as an increase in value at least 15% greater than the previous value of the test. Sixty-five percent (65%) or 45/70 of patient samples with a positive change correlated with disease progression while sixty-one percent 61% or 184/304 of patient serial samples with no significant change in CA 19-9 value correlated with no progression. The total concordance was sixty-one percent (61% or 229/374).

Conclusion: The Lumipulse G CA19-9-N assay was demonstrated to be accurate, precise, and sensitive for the quantitative determination of CA 19-9 antigen in human serum and plasma and is useful in monitoring the course of disease in patients with cancer of the exocrine pancreas.

B-336

Determination of Catecholamine and Metanephrines in Urine by SPE and LC-MS/MS

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Background: Catecholamines are hormones made by the adrenal glands and metanephrines are the metabolites of catecholamines. They are biomarkers of several kinds of tumors, such as pheochromocytoma tumors, paraganglioma tumors and neuroblastoma tumors. These tumors produce excess catecholamines which metabolize to metanephrines. Urinary catecholamines and metanephrines are tested for diagnosis of these tumors. Cation-exchange solid phase extraction (SPE) method removes all neutral and acidic interference in the urine samples. LC-MS/MS is highly sensitive and selective. We developed SPE and LC-MS/MS methods to quantify urinary catecholamines and metanephrines. **Methods:**Human urine samples were fortified with standards (norepinephrine, epinephrine, dopamine, metanephrine and 3-methoxytyramine). Acidified urine was hydrolyzed for 30 minutes. Agility Deluxe WCX plate (30mg/well) was pre-conditioned with 1 ml of acetonitrile, then 1 ml of 50 mM NH4AC. Hydrolyzed urine solution (0.3 mL) was loaded to SPE, then washed with 1 ml each of 10% methanol and IPA. Analytes were eluted with 1 ml of 5% formic acid in acetonitrile, and then evaporated and reconstitute in 10% methanol with 0.1% formic acid. LC-MS/MS method used an ExionLC-API4500 Triple Quad MS Shimadzu HPLC system operated in MRM positive mode. Reliasil C18 column was used for separation with methanol and water gradient mobile phases. LC run time is 5 minutes.

Results:A couple of HPLC columns have been tested to separate catechoamines and metanephrines. As they are relative polar, a lower carbon loading C18 column gives better retentions. LC-MS/MS method is using Reliasil C18 column with gradient 0.1% formic acid in water to 50% 0.1% formic acid in methanol in 5 minutes. The linearity of norepinephrine is 10-200 ng/mL, 5-200 ng/mL for epinephrine, 10-1000 for dopamine, 20-1500 for metanephrine, and 30-200 for 3-methoxytyramine. The recoveries of these compounds are in the range of 86-99% with CV% of ± 11% . **Conclusion:**Based on study results, the method will be further tested using 30 mg/cc 96-well plate. The extraction method will also be modified to accommodate the WCX micro-elute plate, to further reduce the sample size and organic solvents usage.

B-338

Validation of an In-House Assay for BRCA2 c.156_157insAlu Screening in Brazilian Patients

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Background: Breast Cancer is the most common cancer in women worldwide. Around 5-10% of breast and 10-15% of ovarian cancers are a result of mutations in *BRCA* genes. The investigation of *BRCA1* and *BRCA2*-associated with hereditary breast and ovarian cancer (HBOC) in specific populations allows the identification of founder effects. *BRCA2* c.156_157insAlu has been reported as a founder mutation from Portuguese population, representing 58% of all germline deleterious *BRCA2* mutations observed. This pathogenic insertion causes the *BRCA2* exon 3 skipping. Since Alu elements are around 300-350 bp long, they may not be efficiently detected by NGS or MLPA techniques. Furthermore, those are expensive approaches. **Objective:** To validate a simpler, cost-effective and reliable assay for *BRCA2* c.156_157insAlu screening in Brazilian patients. Genomic DNA was extracted from whole blood samples using Gentra® Puregene® Blood Kit (QIAGEN). A two-step PCR was performed for *BRCA2* exon 3 and Alu insertion, according to Machado and colleagues (2007): (1) PCR with primers 3F/3R; and (2) nested PCR with primers 3AluF/3AluR. Different PCR conditions (DNA input, Taq DNA Polymerase, annealing temperature) were tested. The PCR products were analyzed by agarose gel electrophoresis. Ten samples (one c.156_157insAlu-positive, four c.156_157insAlu-negative and five unknown) were used for assay validation. Inter-laboratory validation was performed for five samples. Assay performance was further evaluated for twenty-seven patients with a decrease in peak signal (< 0.79 ratio) in exon 3 for SALSA MLPA probemix P045 BRCA2/CHEK2 (MRC-Holland). This decrease could be due to a single-exon deletion or variants that modify the reference sequence detected by MPLA probes, including Alu insertion in exon 3. **Results:** The DNA input ranged from 50 to 400 ng. No optimal amount was observed. Taq DNA Polymerase recombinant (Invitrogen) presented better performance than Taq Platinum (Invitrogen). The optimal primer annealing temperature was 65ºC. Inter-laboratory comparison showed 100% concordance. From the 27 MLPA-inconclusive results, nine (33.3%) were positive for *BRCA2* Alu insertion. **Conclusion:** Screening of founder mutations is more cost-effective than screening the whole gene, making it possible to use more specific approaches for molecular testing. The *BRCA2* c.156_157insAlu mutation is one of the most common variant identified in Portuguese HBOC families. However, it is still rare in Brazilian population. The investigation of this rearrangement in countries with Portuguese ancestry (e.g. Brazil) is strongly recommended.

B-339

Frequency of Variants in the *BRCA1* and *BRCA2* Genes in Patients without a Family History of Breast and/or Ovarian Cancer

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Background: Variants in different genes are known to cause susceptibility to breast and / or ovarian cancer. Approximately 10-20% of high-grade ovarian cancers are associated with variants in the *BRCA1* and *BRCA2* genes. Currently, the search for variants in these genes is mostly prescribed by geneticists based on the National Comprehensive Cancer Network (NCCN) guidelines for patients with a family history of cancer and for genetic counseling. **Objective:** This study aims to assess the frequency of patients who have variants in the *BRCA1/2* genes, but do not have a family history of cancer.

Methods: A total of 480 patients with ovarian cancer were selected and screened for germline variants in *BRCA1/2* genes by next generation sequencing (NGS) using amplicon methodology. **Results:** A total of 121 (25.2%) patients presented variants in the BRCA1/2 genes. Among these, 77 showed variants in *BRCA1* distributed as: pathogenic (59), probably pathogenic (6) and VUS (12). In *BRCA2*, 44 variants were identified and classified as: pathogenic (21), probably pathogenic (2) and VUS (21). After family history evaluation, we found 72 (59.5%) patients with variants in the *BRCA1* and *BRCA2* genes which have a relevant family history of cancer and 49 (40.5%) without family history. From the 31 (63.3%) patients with variants in the *BRCA1* gene and without a family history, 23 were classified as pathogenic or probably pathogenic, while for the 18 (36.7%) patients with variants in *BRCA2*, five were classified as pathogenic or probably pathogenic. In contrast, of the 359 (74.8%) patients with no variants detected, more than 50% had a cancer family history. **Conclusion:** This survey reveals the importance of evaluating additional inclusion criteria, other than familial cancer history, for the indication of molecular tests, such as the sequencing of BRCA1 and BRCA2 for breast and ovarian cancer.

B-342

QIP-Mass Spectrometry in High Risk Smoldering Multiple Myeloma Patients included in the GEM-CESAR Trial: Comparison with Conventional IMWG Response Assessment

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Background:

The GEM-CESAR trial is a potentially curative strategy for high-risk smoldering multiple myeloma (HRsMM) patients in which the primary endpoint is the assessment of bone marrow minimal residual disease (MRD) negativity by next generation flow. The technique is highly sensitive and provides prognostic information but is an invasive procedure. We have evaluated quantitative immunoprecipitation mass spectrometry (QIP-MS), a polyclonal antibody-based technology to identify monoclonal immunoglobulins in serum, as an alternative method of tumor burden.

Methods:

Ninety HRsMM patients included in the GEM-CESAR trial received six 4-week cycles of carfilzomib, lenalidomide and dexamethasone followed by high dose melphalan and ASCT and 2 further cycles of consolidation with the same regimen. All patients received maintenance with lenalidomide up to 2 years. Serum protein electrophoresis (SPEP) and immunofixation (IFE) were performed using standard procedures. QIP-MS assessment has been previously described (1). Immunoprecipitation with paramagnetic beads allowed us to characterise the isotype of each Ig, and measure the molecular mass of each Ig for each specific patient, with enough precision and accuracy to establish clonality. Standard response assignment was carried out as per the international myeloma working group (IMWG) guidelines.

Results:

We confirmed the higher sensitivity of QIP-MS to identify the M-protein in serum compared to conventional electrophoretic methods. QIP-MS was 100% concordant with IFE for isotyping the M-protein in baseline samples (41 IgGκ, 16 IgGλ, 13 IgAκ, 13 IgAλ, 4 κ FLC, 3 λ FLC). In 5 samples QIP-MS identified additional monoclonal proteins that were not detected by IFE. Furthermore, a glycosylated M-protein was detected by mass spectrometry in 7 cases. The percentage of patients with detectable disease post-induction, post-transplant and post consolidation was 58% (51/88), 36% (30/83) and 24% (20/83) by SPEP/IFE and 79% (60/76), 55% (42/76) and 53% (41/77) by QIP-MS respectively. The overall concordance between QIP-MS and SPEP/IFE at the three timepoints was 76.3% post induction (n=76), 75.3% post-transplant (n=73) and 67.1% post-consolidation (n=76). No patients were identified positive by SPEP/IFE and negative by QIP-MS. By contrast amongst CR patients, QIP-MS identified residual M-protein in 60% (18/30) patients post-induction, 38% (18/47) post-ASCT and 43% (25/58) post-consolidation.

Conclusion:

QIP-MS shows greater sensitivity than standard electrophoretic methods for the detection of M-proteins, enabling better characterisation of patients during routine screening. The ability to measure a unique molecular mass for any M-protein offers improved specificity of patient monitoring. QIP-MS can detect residual disease in patients achieving a standard CR and therefore could be complementary to bone marrow MRD assessment.

1. North S, Barnidge D, Brusseau S, Patel R, Haselton M, Du Chateau B, et al. QIP-MS: A specific, sensitive, accurate, and quantitative alternative to electrophoresis that can identify endogenous m-proteins and distinguish them from therapeutic monoclonal antibodies in patients being treated for multiple myeloma. Clinica Chimica Acta 2019;493:S433.

B-344

QIP-Mass Spectrometry in Functional High Risk Multiple Myeloma: Comparison with Conventional Response Assessment

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Background: Multiple myeloma (MM) is a heterogenous disease characterised by the presence of multiple clones. Immunoenrichment-coupled mass spectrometry provides high analytical sensitivity and allows individual clone-tracking over time. We have evaluated quantitative immunoprecipitation mass spectrometry (QIP-MS) for the identification of monoclonal immunoglobulins in a uniformly treated cohort of functional high-risk MM patients.

Methods: Fifty newly diagnosed MM patients failing front-line bortezomib-based induction therapy were enrolled onto the Australasian Leukaemia and Lymphoma Group (ALLG) MM17 trial (ACTRN12615000934549) evaluating a combination of carfilzomib, thalidomide and dexamethasone. Serum immunofixation (IFE) was performed before and after autologous stem cell transplantation (ASCT) and at the end of consolidation. Response assignment was carried out as per the International Myeloma Working Group guidelines. For QIP-MS studies, polyclonal antibodies (anti-IgG, -IgA, -IgM, -total κ and -total λ light chain, -free κ and -free λ light chain) covalently attached to paramagnetic microparticles were incubated with serum, washed and treated to simultaneously elute and reduce patient immunoglobulins. Light chain mass spectra were generated on a MALDI-TOF-MS system. A positive QIP-MS score in follow-up samples was based on the presence of an M-protein at the same mass as determined in the pre-treatment samples. Matched serum IFE and QIP-MS results were available in 29 patients pre-ASCT, 28 post-ASCT and 20 post-consolidation.

Results: QIP-MS identified the serum monoclonal component at baseline in all patients; in 6 cases the M-protein mass spectrum was consistent with the presence of

glycosylated forms. We confirmed the higher sensitivity of QIP-MS to identify the M-protein compared to conventional electrophoresis during monitoring. The percentage of patients with detectable disease pre-ASCT, post-ASCT and post-consolidation was 76%, 54% and 30% by IFE; and 90%, 54% and 50% by QIP-MS, respectively. Overall there was moderate concordance between IFE and QIP-MS for detecting an M-protein (κ=0.57, 79% agreement). Among the discordant cases 12 IFE-negative patients were positive by QIP-MS. By contrast 4 IFE-positive patients were negative by QIP-MS; in all 4 cases QIP-MS identified an M-protein with a different mass than at baseline but of the same isotype, suggesting that the IFE results in these patients do not correspond with the original clone. Finally, 10/31 (32%) patients in complete response or better (≥CR) (4 pre-ASCT, 2 post-ASCT and 4 post-consolidation) demonstrated the presence of residual M-protein by QIP-MS.

Conclusion: QIP-MS shows greater sensitivity than standard electrophoretic methods for the detection of M-proteins, enabling better characterisation of patients during routine monitoring. The ability to measure a unique molecular mass for any M-protein offers improved specificity of patient monitoring. QIP-MS can detect residual disease in patients achieving a standard CR and therefore could be complementary to bone marrow assessment.

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Dosages of Tumor Markers with Higher Demands for Automated Biochemical Equipment for Clinical Laboratory

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Background: The measurement of tumor markers (TM) provided by clinical laboratories can be useful in the management of cancer patients, assisting in the processes of diagnosis, staging, evaluation of therapeutic response, detection of relapses and prognosis, due to the direct correlation between the change in levels of these markers with clinical status[1,2]. The results of TM tests by different methods are not harmonized, therefore, the values obtained by different methods cannot be used interchangeably. In the change of analytical platform, educational measures and dissemination to the clinical staff it´s necessary to previously change to minimize the impact on the patient´s clinical follow-up. If during the monitoring of a patient there is a change in the test method used to determine the serial levels of TM, the laboratory should inform the clinician of the need to restart monitoring[3]. In this study, the authors aimed to evaluate the proposed TM for the change of platform and the impacts in a large clinical laboratory in Brazil. **Methods:** The assays were evaluated: CEA, CA 15-3, CA19-9, CA125, PSA and free PSA on the Atellica IM Siemens Healthineers Analyzer. Evaluation Verification of Precision and BIAS estimation peer groups were performed through the repeatability ($\%CV_R$) and within-laboratory precision ($\%CV_{WL}$), according to EP15-A3; with a total of 25 samples per quality control (QC) level. The coefficient of variation (CV%) obtained was compared with the manufacturer's and the analytical quality specifications (TEa) metrics. Comparison studies were performed between the Atellica IM 1600 Analyzer and Architect Abbott according to EP09, using at least 40 serum samples. For the evaluation of the Sigma's Metric, the precision and bias components were used for each level of QC. The first choice of the TEa's metric for the proposed study was based on the VB2014. **Results:** The results obtained in the studies reached and exceeded the specification goals. The $\%CV_{WL}$ were from 2.6% to 6.6% and the BIAS from 1.39% to 11.60% and all QC levels obtained a Sigma's metric greater than 3 , with 8 out of 12 levels with results 6 Sigma (world class) and 2 out of 12 with results above 4 Sigma (good) and 2 out of 12 above 3 Sigma (desirable). In the comparison study, R^2 were 0.99 to 1.0 for all TM showing excellent correlation, despite the excellent correlation. **Conclusion**: For the CA 125 and CA 19-9 assays, special attention is needed in the implementation with the clinical staff, due to the BIAS of -24.57% and -43.91%, TE 29.36% (TEP=35.36) and 53.68% (TEP=46.07%) respectively, however when evaluated through Sigma's metrics with peer group, these assays presented world class performance (6 sigma and up) suggesting the existence of methodological differences. The assays tested on Atellica IM demonstrated excellent analytical performance. Imprecision and BIAS are consistent and demonstrate acceptable Sigma levels, like those found in previous studies and can provide a basis for understanding the quality performance of the Atellica Solution tumor marker assays. **Siemens Healthineers supported the studies by providing systems, and reagents.*

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Detection of Stage 0 ~ I Lung Cancer by CYFRA 21-1-Anti-CYFRA 21-1 Autoantibody Immune Complex and CYFRA 21-1 Ratio

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Background: Lung cancer is the most common cancer and the leading cause of 18.4% of the total cancer deaths. In 2018 alone, about 2 million people were diagnosed with lung cancer, and approximately 1.7 million deaths were associated with lung cancer on a global scale. The asymptomatic nature of lung neoplasms is the reason behind the diagnosis at an advanced stage when cure with current therapies is unlikely. Even though the 5-year survival rate in lung cancer is low, patients with early-stage have a higher survival rate as compared to later localized stage and metastasized stage (54.8% vs. 27.4% vs. 4.2%, respectively). It is known that the screening and identification of lung cancer at an early stage saves lives. Hence, detection of lung cancer at stage 0 ~ I in a population-based screening is a public health priority. The tumor marker-specific autoantibodies show 5-10 times higher levels in cancer patients than in a healthy population. **Method:** We hypothesized that plasma levels of CYFRA 21-1-Anti-CYFRA 21-1 autoantibody immune complex (CIC) would be at higher levels compared to the free CYFRA 21-1 in lung cancer patients than in healthy population. Whereas, the plasma levels of CIC will be at lower levels compared to the free CYFRA 21-1 in a healthy population than in lung cancer patients. Here, we describe the development of a method for the detection and quantification of free CYFRA 21-1 (0.05 - 5ng/mL) and CIC (0.05 - 5ng/mL) in the plasma samples. About 120 samples from healthy control and 50 samples from the lung cancer patients were used for the evaluation of the developed method. **Results:** The sensitivity and specificity for the detection of lung cancer were found to be 76.0% (95% CI, 61.8 - 86.4) and 87.5% (95% CI, 80.2-92.8) when CIC/CYFRA 21-1 ratio was used. By

using CIC, the sensitivity and specificity were 66.0% (95% CI, (51.2 - 78.8) and 61.7% (95% CI, 52.3 - 70.4), respectively. Whereas, when only CYFRA 21-1 was used, the sensitivity and specificity were 32.0% (95% CI, 19.5 - 46.7) and 59.2% (95% CI, 49.8 - 68.1), respectively. The sensitivity for stage I lung cancer detection by using CIC/CYFRA 21-1 ratio was found to be 76% (95% CI, 54.8 - 90.5). **Conclusion:** The obtained results indicate that the CIC/CYFRA 21-1 ratio can aid in the detection of stage 0 ~ I lung cancers. Therefore, the CIC/CYFRA 21-1 ratio can be applied for the identification of asymptomatic patients in a seemingly healthy population.

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Lung Cancer Index Test for the Detection of Stage 0 ~ I Lung Cancer with High Sensitivity and Specificity

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Background: About 2 million people were diagnosed with lung cancer in 2018 alone, and approximately 1.7 million people died due to lung cancer on a global scale. The early-stage lung neoplasms are asymptomatic in nature that leads to the diagnosis of lung cancer at an advanced stage when it is difficult to cure with current therapies. The patients with early-stage have a higher survival rate as compared to the later localized stage and metastasized stage (54.8% vs. 27.4% vs. 4.2%, respectively). Therefore, it is crucial to detect lung cancer at stage 0 ~1 with very high sensitivity and specificity to saves lives. The identification of stage 0 ~ I (asymptomatic) cancer patients from a healthy population remains a challenge. Therefore, a cancer screening test that is easy to implement and show high specificity and sensitivity are crucial for the identification of stage 0 ~ I cancer patients from a healthy population. **Method:** A Lung Cancer Index (LCI) was developed for the identification of stage 0 ~ I (asymptomatic) cancer patients from the healthy population. The LCI is measured by using the levels of biomarkers such as cTnT, NT-proBNP, CYFRA 21-1-Anti-CYFRA 21-1 autoantibody immune complex (CIC), and CYFRA 21-1. About 120 samples from healthy control and 50 samples from the treatment-naïve stage 0 ~ IV lung cancer patients were used for the evaluation of the developed method. **Results:** The levels of cTnT, NT-proBNP, CYFRA 21-1-Anti-CYFRA 21-1 autoantibody immune complex (CIC), and CYFRA 21-1 were measured by using 9G test™ Cancer/Lung, a lung cancer identification test. The cTnT/NT-proBNP ratio, CIC/CYFRA21-1 ratio, and LCI (cTnT/NT-proBNP x CIC/CYFRA21-1) were used for the determination of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) for the detection of stage 0 ~ IV lung cancer. The sensitivity and specificity for the detection of lung cancer were found to be 84.0% (95% CI, 70.9 - 92.8) and 87.5% (95% CI, 80.2 - 92.8) when cTnT/NT-proBNP ratio was used. The sensitivity and specificity for the detection of lung

cancer were found to be 76.0% (95% CI, 61.8 - 86.9) and 84.2% (95% CI, 76.4 - 90.2) when CIC/CYFRA21-1 ratio was used. The sensitivity and specificity for the detection of lung cancer were found to be 76.0% (95% CI, 61.8 - 86.9) and 97.0% (95% CI, 91.7 - 99.1) when LCI was used. The specificity for the detection of lung cancer was increased from 84.2% to 97.0% by determining the LCI. Moreover, the accuracy was found to be increased from 81.8% to 90.6%.

Conclusion: This study provided proof that a Lung Cancer Index obtained by cTnT/NT-proBNP x CIC/CYFRA21-1 can identify stage 0 ~ IV lung cancer with 76% sensitivity and 97.0% specificity. Therefore, the Lung Cancer Index can be applied for the identification of stage 0 ~ I lung cancer patients in a seemingly healthy population.

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Development of PIVKA-II Assay for Fully Automated Analyzer LUMIPULSE® G1200 based on PIVKAII Molecular Character Analysis

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Background:Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is an abnormal type of prothrombin, and it has been well known to be elevated in a patient with hepatocellular carcinoma (HCC) or the lack of vitamin K. Clinical utility of PIVKA-II in HCC patients has been established by various study using MU3 monoclonal antibody which recognize γ-carboxyglutamic acid residues (Gla) and glutamic acid residues (Glu) domain that is PIVKAII specific region in N-terminal. We have analyzed the PIVKA-II molecule in various specimens, and found out the diversity of PIVKA-II molecule by a hydrophobic interaction chromatography (HIC) analysis. In this study, to reduce the difference of PIVKA-II immunoreactivity among PIVKA-II molecules with different hydrophobicity, we selected two kinds monoclonal antibodies and developed the new PIVKAII immunoassay on the fully automated chemiluminescent enzyme immunoassay (CLEIA) system (LUMIPULSE® G1200 system, FUJIREBIO INC.) and examined the fundamental performance.

Methods:We investigated the analysis of PIVKAII molecules to characterize molecular forms and diversity in various specimens including serum and plasma. We evaluated the combination of MU3 monoclonal antibody for the solid phase and several monoclonal antibodies for the tracer, and then, we developed Lumipulse G PIVKAII-N reagent by selecting two kinds of monoclonal antibodies for the conjugate. Fundamental performance testing was performed. Correlation with commercial available reagents, matched pair correlation between serum and plasma were evaluated following recommendation from CLSI documents. All evaluations were executed with LUMIPULSE G1200 instrument.

Results: On HIC analysis, there were observed several elution patterns of PIVKA-II immuno-reactivity with several antibodies, indicating such as two peaks on the higher and the lower hydrophobic fractions, or only one peak with higher hydrophobic fractions. In addition, each antibody showed the different immune-reactivity to both fractions. Therefore, we determined to choose the combination of monoclonal antibodies to react to both fractions equivalently for Lumipulse G PIVKAII-N. As a result of fundamental performance for Lumipulse G PIVKAII-N, within-run precision % CVs for our assay ranged from 1 to 3% when 3 different conc. of quality controls were tested. Correlation between serum and heparin plasma and EDTA plasma with 60 matched pair samples was excellent (slope: 1.01, regression: 0.999). The significant correlation with Picolumi PIVKA-II mono using 60 specimens was observed (slope: 0.99, regression: 0.978).

Conclusion:Our study indicates that PIVKA-II molecules in specimens possess the different character of hydrophobicity. We focus on this different character and developed a new PIVKAII assay that can react equally to different hydrophobic molecules on LUMIPULSE G1200 instrument.

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Comparison of Monoclonal Gammopathy Assessed by Mass Spectrometry vs. Serum Protein Immunofixation in Peripheral Blood in Multiple Myeloma

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Background: Serum protein electrophoresis (SPEP) and immunofixation (IFIX) have remained the standard-of-care for detection of monoclonal paraprotein (MP) in the peripheral blood (PB). However, this test can result in false negative results on account of the insensitivity of this assay as well as interrater variability. Mass spectrometry as performed by both MALDI-TOF and liquid chromatography MS (LCMS) can also be used to detect MP. We assessed the concordance between SPEP/IFIX and MS in PB of patients with multiple myeloma (MM) at diagnosis and after completion of intensive therapy.

Methods: Paired PB samples were evaluated from pts with newly diagnosed secretory MM who received Carfilzomib/Lenalidomide/Dexamethasone (KRd) for 4 cycles, melphalan 200 mg/m^2 with autologous stem cell transplant (ASCT), KRd for 14 cycles post-ASCT, and lenalidomide maintenance until progression. Both SPEP/IFIX and MS were evaluable in 36 pts at diagnosis and after 18 cycles of KRd (C18), and in 24 pts after 1 year of maintenance. Polyclonal antibodies (anti-IgG, -IgA, -IgM, -total κ and -total λ light chain, -free κ and -free λ light chain) covalently attached to paramagnetic microparticles were incubated with serum, washed and treated to simultaneously elute and reduce patient immunoglobulins. Light chain mass spectra were generated on MALDI-TOF-MS and LCMS systems. MS signatures for clone-tracking were defined at baseline based on the unique molecular mass of the M-protein. SPEP/IFIX was interpreted by local pathologist.

Results: A MP was identified at diagnosis in all 36 patients by both IFIX and MALDI-TOF/LC-MS. In 2/36 (6%) cases, MS identified an additional clone that was not detected by IFIX at diagnosis. There was fair concordance between IFIX and MALDI-TOF among the 60 on-treatment samples (κ=0.367,68% agreement); 14 IFIX$^+$/MALDI$^+$, 27 IFIX$^-$/MALDI$^-$, 3 IFIX$^+$/MALDI$^-$ and 16 IFIX$^-$/MALDI$^+$. There was slight concordance between IFIX and LCMS (κ=0.196, 52% agreement); 16 IFIX$^+$/LCMS$^+$, 15 IFIX$^-$/LCMS$^-$, 1 IFIX$^+$/LCMS$^-$ and 28 IFIX$^-$/LCMS$^+$. The three patients with discordant IFIX$^+$/MALDI$^-$ and one patient with discordant IFIX$^+$/LCMS$^-$ were all found to have a different clone of the same isotype by MS, suggesting the IFIX$^+$ results in these patients do not correspond with the original clone at diagnosis.

Conclusion: Detection of MP by MS has superior sensitivity compared to SPEP/IFIX during follow-up of patients with secretory multiple myeloma. MS at diagnosis may assist in identifying multiple clones that can be tracked over time to assess minimal residual disease.

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Feasibility Study of NuProbe VarTrace AML Multi-Gene Panel

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Background: For Acute Myeloid Leukemia (AML) patients, the National Comprehensive Cancer Network (NCCN) recommends molecular diagnostic testing for several genes including FLT3, IDH1, IDH2, DNMT3A, KIT and NPM1. The presence of specific mutations in these genes in AML patients can guide treatment with targeted therapies such as RYDAPT® (midostaurin), XOSPATA® (gilteritinib), TIBSOVO® (ivosidenib), and IDHIFA® (enasidenib). The objective of this study is to perform a feasibility study of the VarTrace AML Multi-gene Panel from the NuProbe in our molecular oncology laboratory at Versiti BloodCenter of Wisconsin.

Methods: NuProbe's VarTrace AML Multi-gene Panel is designed to detect and quantify somatic mutations (including 39 hotspot mutations) in genes FLT3, DNMT3A, IDH1, IDH2, KIT, and NPM1 that are of clinical relevance for AML. The kit uses NuProbe's PCR-based Blocker Displacement Amplification (BDA) method to enable the selective amplification of low-abundance sequence variants (single nucleotide variants and insertions/deletions) in a background of wildtype DNA. Following PCR enrichment, Sanger sequencing is applied to reveal the identity of the variant, thereby improving the sensitivity of Sanger sequencing more than 100 fold.

We obtained the VarTrace AML Multi-gene Panel kit from NuProbe and performed the Real-Time quantitative PCR amplification using Roche LightCycler 480 instrument. Eight reactions are required for each sample and control. Reaction 1-7 is used for selective variant enrichment, and reaction 8 is used for input quantification and normalization. The sample Cq value is defined as the difference in Ct values of reaction 1-7 and reaction 8, (i.e., Cq1 = Ct_1 - Ct_8, Cq2 = Ct_2 - Ct_8, etc.). Cq values and sample variant allele frequency (VAF) are expected to have a log linear correlation, which is used to establish calibration for quantifying sample VAF. If the sample Cq value is more than 1 cycle less than negative control Cq value, then the variant is called positive followed by sequence verification by Sanger Sequencing and variant allele frequency (VAF) quantification calculator provided with kit. If Cq value difference is less than 1 cycle, the variant is called as wild-type at the specific gene loci. In our laboratory, we performed the experiment of NuProbe AML Multi-gene Panel using positive control (PC), negative control (NC) and no template control (NTC).

Results: The results of the controls were found as expected. The positive control identified the expected specific somatic mutations with high sensitivity and low variant allele frequency ranging from 0.05% to 2.23% (FLT3, p.D835H: 0.73%; DNMT3A, p.R882C: 0.94%; IDH1, p.R132C: 0.42%; IDH2, p.R140W: 1.11%; IDH2, p.R172K:

2.23%; KIT, p.D816H: 0.05%; NPM1, p.W288fs*12: 0.24%) and variant allele peaks are also apparent in Sanger traces of targeted amplicons. We hope to enhance the VAF of the expected specific somatic mutations further in the future.

Conclusion:

Based on the feasibility study, our laboratory has shown detection of clinically actionable specific mutations in AML is achievable. It will help our laboratory in validating a high sensitive and quantitative AML multigene panel as a clinical test for early detection of AML in patients with low tumor burden and provide targeted therapies.

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Evaluation of Cell-Free DNA Isolated from Urine and Plasma using JBS Science Technology for Detection of Circulating Tumor DNA

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Background: Liquid biopsies using urine and blood offer a minimally invasive approach for sampling cell-free circulating DNA (cfDNA) for early detection of cancer mutations, to monitor therapeutic responses, and to capture a 'global' portrait of tumor heterogeneity. Today, the study of cfDNA is challenging due to low cfDNA yields, high cfDNA fragmentation, and cell-associated genomic DNA contamination. Thus, optimizing cfDNA isolation to obtain high quality and quantity for downstream analysis is very important. We report a magnetic-bead based method for cfDNA isolation from urine or plasma using the JBS platform with semi-automation capabilities that offers improved or highly comparable cfDNA yields and quality compared to commercially available column and bead-based methods. Performing the JBS DNA clean-up step to cfDNA samples that were isolated with commercially available methods improved downstream assay performance.

Methods: To evaluate the JBS cfDNA isolation method, urine (n=6) and plasma (n=6) samples were collected or purchased commercially from donors and spiked with a synthetic 141bp double-stranded DNA fragment. Thereafter, cfDNA was isolated from urine (3mL) or plasma (2mL) in triplicate using three kits, the JBS urine/plasma cfDNA kit, a column-based kit (Kit Q), and a bead-based kit (Kit M) according to the manufacturer's protocol. We assessed recovery of cfDNA and the synthetic fragment by TapeStation and quantitative real-time PCR (qPCR), respectively, and we measured protein carryover with the Qubit protein assay kit. We tested the hypothesis of no difference among kits in recovery of cfDNA and synthetic spike-in DNA using analysis of variance (ANOVA) or a Kruskal-Wallis test when parametric assumptions were not met. Tukey's post hoc comparisons were used for ANOVA procedures and Mann-Whitney U Pairwise comparisons for Kruskal-Wallis procedures.

Results: Fragment size profiles of cfDNA isolated from urine and plasma by all three kits were comparable and exhibited distinct mono-nucleosome sized peaks in the 150-170bp range. In urine samples, the three kits differed significantly in recovery of the synthetic DNA fragment as measured by qPCR (χ^2= 11.55, df= 2, p= 0.003). The JBS kit recovered significantly more synthetic DNA than did Kit Q (p= 0.023) or Kit M (p= 0.004). In plasma samples, analyses suggested there were differences in the spike-in recovery rate (χ^2= 7.52, df = 2, p= 0.023), however post hoc comparisons were not significant. We detected carryover protein in plasma cfDNA isolated by Kit Q ranging from 8.7-28.9µg/mL, but not in plasma cfDNA isolated with the JBS kit or Kit M. Protein was not detected in any urine isolates. We performed the JBS clean-up method on cfDNA isolated with Kit Q and Kit M. Afterwards, carryover proteins were undetectable from Kit Q plasma isolates and recovery of spike-in DNA from both urine and plasma increased as measured by qPCR (p≤ 0.006), suggesting impurities (e.g., PCR inhibitors) were removed.

Conclusion: Assessment of cancer patient urine and plasma for circulating tumor DNA detection is ongoing. The JBS cfDNA isolation method exhibits high cfDNA yield and quality and can be semi-automated to facilitate the extraction of cfDNA from liquid biopsies in an efficient manner for cancer research.

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Evolution and Development of an Automated Finger Prick Gene-Based Diagnosis for the Neuroendocrine Genotype

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Neuroendocrine tumor disease represents 1-2% of all cancers and is considered an "orphan" disease. Tumors are difficult to diagnose and there are no reliable biomarkers for clinical management. In the 1960's, diagnosis was histopathological and based on tumor tissue from surgery or biopsy. The development of antibodies against specific neuroendocrine cell products like chromogranin A in the 1970s provided the basis for immunohistochemical assessment of disease and the development of mono-analyte blood ELISAs and RIAs. Measuring secretory products, however, is not sensitive. The introduction of molecular strategies (1990-2000) based on genomic sequencing, transcriptomics and gene expression assays revolutionized cancer biology and refocused investigation on pathobiologically important pathways and hallmarks of disease. This led to the development of clinically useful tissue-based tools e.g., 72-gene PCR-based Mammaprint assay, which has recently been validated (2018) for breast-cancer treatment decision-making. More recently, the focus has switched from invasive strategies to blood-based tools with the development of assays that detect treatment-relevant mutations in circulating tumor DNA e.g., EGFR L858R and second-line treatment (TARCEVA) decisions in lung cancer. In 2005, we defined transcriptome profiles of gastrointestinal and pancreatic NETs and in 2010, developed molecular-based tissue classifiers utilizing machine learning and multigene analyses. Although this strategy was >95% accurate for diagnosis, it still relied on tissue. Between 2010 and 2015 we investigated if blood was an alternative compartment for NET gene identification. Our studies identified 51 genes that were co-expressed (R>0.8) in tissue and blood. Scores derived from genes co-expressed in blood and tissue were equivalent and provided the basis for a "liquid" biopsy (NETest). Metrics were >90% sensitivity and specificity. Clinical evaluation (n>6,500 patients) demonstrated the multigene assay to have clinical utility as a prognostic biomarker. These gene expression features have recently (2018) been validated in a large TCGA-dataset. In 2019, to facilitate measurement, the assay was automated using targeted PCR and spotted plate technology with significant concordance (p<0.0001) between standard qPCR (R>0.95; n=280 NETs, n=125 controls). To obviate the cold-chain problem related to mRNA stabilization in blood samples, we developed an RNA stabilization buffer that maintains NETest signature integrity at room temperature for up to 10 days. Comparison of 120 matched samples show significant concordance in NETest levels (R>0.93). To move beyond venipuncture, we miniaturized the assay and developed a 50ul of blood finger-prick micro-assay system which is concordant (n=50, R>0.95) with venipuncture. Most recently, we have determined that the NETest can function as an accurate (>90%) tool to diagnose neuroendocrine-like differentiation (NELD) in other cancers that develop resistance to standard therapies e.g., ADT and castration-resistance in prostate cancers. We have defined molecular genomic signatures for the neuroendocrine genotype/phenotype in blood and demonstrated that a NET gene expression assay in blood is accurate and can be automated and miniaturized. The implications for facilitation of diagnosis and management of NETs and tumors that develop NELD-features warrant investigation.

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Measuring Secreted Proteins Versus Tumor-Derived mRNA in Blood: Comparative Evaluation of Chromogranin A Versus the NETest as Neuroendocrine Tumor Diagnostic Markers

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Background:

Chromogranin A (CgA) is a commonly used diagnostic and monitoring tool for neuroendocrine tumor disease despite NCCN guidelines identifying it as a category 3 (major disagreement about utility) biomarker. Several commercial assays have been developed to measure this protein (or its fragments) and are available both at CLIA-certified laboratories (USA) as well as in NET Centres of Excellence (CoEs - Europe). CgA is typically reimbursed by insurance companies and appears in several guidelines (e.g., ENETs). We sought to directly evaluate the accuracy of detecting NET disease using two different CgA assays, one in the USA (NEOLISA, EuroDiagnostica, IBL-America, CLIA-certified laboratory) and one in an ENETS CoE (CgA ELISA Demeditec Diagnostics, Germany). We compared the results to the NETest, a circulating mRNA assay, recently validated as an *IVD* for NETs.

Methods:

NETs (*n*=258) including lung: *n*=43; duodenum *n*=9; gastric: *n*=44; pancreas: *n*=67; small bowel: *n*=40; appendix: *n*=10; rectum: *n*=45. One hundred and twenty-two had no image-evidence of disease (IND) while 136 had image-positive disease (IPD).

CgA assays (plasma): NEOLISA, ULN >108ng/ml (inter-assay CV: 13.6%, intra-assay CV: 11.2%), DD: ULN>99ng/ml (inter-assay CV: 10%, intra-assay CV: 9%). Data mean±SEM.NETest (whole blood): 51-marker genes, pre-spotted plates, qRT-PCR, normalization and multianalyte algorithmic analyses (inter-assay CV: 4.2%, intra-assay CV: 1.3%), CLIA-laboratory. All samples were blinded.

Statistics: Mann-Whitney U-test, Pearson correlation & McNemar-test, AUROC analyses.

Results:

In the whole group (*n*=258), CgA levels were significantly ($p<0.0001$) higher with the NEOLISA assay (216±91ng/ml) vs. the DD-assay (76±8ng/ml). The assays exhibited a high concordance in output (Pearson $r=0.81$, $p<0.0001$), but there were 10.9% (*n*=31) discordant results. This reflected the NEOLISA assay detecting more samples as CgA-positive.

IPD group: CgA-positives were detected in 48/136 (35%, NEOLISA) vs. 28 (21%, DD-assay). McNemar's $Chi^2=15.04$, $p<0.001$ OR: 11.0, identifying the NEOLISA was significantly better than the DD-assay. In the IPD cohort, the NETest, in contrast, was positive in 135 (99%; OR: 87-106, $p<0.0001$).

IND group: CgA-positives were detected in 12/122 (10%, NEOLISA) vs. 9 (7%, DD-assay; *p*=NS). The majority (75%) of positives were associated with gastric NETs consistent with ECL cell hyperplasia. The NETest was positive in 7 (6%); 4 were gastric NETs and 3 exhibited elevated CgA.

AUROC analyses: The AUCs for NEOLISA was 0.65±0.04, DD: 0.61±0.03 and NETest was0.91±0.02. There were significant differences between the NETest and NEOLISA (z-statistic 6.87, $p<0.0001$) and DD (z-statistic 8.04, $p<0.0001$) as well as between the two ELISA assays (z-statistic 2.06, $p=0.039$).

Conclusion:

Two standard CgA assays used for NET management (one accepted by Medicare in the USA, the second used at a CoE in Europe) only detect image-detectable NET disease in 21-35% of cases. Despite measuring the same secretory product, the two CgA assays were significantly different. In contrast to measurements of a protein, a circulating mRNA fingerprint, the NETest, is ~99% accurate for detecting neuroendocrine tumors. Measuring tumor-derived mRNA therefore affords a significantly more sensitive and accurate tool for NET diagnosis than measuring secreted proteins.

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The Hydrashift 2/4 Daratumumab Assay: Our Experience in a High-Volume Cancer Center

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Background: The Hydrashift 2/4 Daratumumab Assay is an FDA approved method used to mitigate daratumumab interference in immunofixation electrophoresis (IFE). This assay is a modified IFE procedure that uses anti-daratumumab antisera to shift the migration of daratumumab on the gel allowing a patient's M-protein to be viewed without interference. The objectives of this study were the following: 1. Determine the magnitude of daratumumab interference in serum protein electrophoresis (SPEP). 2. Determine when the patient's IFE would transition from a true positive, due to M-protein, to a false positive, due to daratumumab only. 3. Formulate a workflow for the appropriate use of the assay in patients who are receiving daratumumab-based therapy.

Methods: A retrospective review of Hydrashift results, corresponding SPEP and IFE results, and patient charts was performed. 38 multiple myeloma patients undergoing daratumumab-based therapy were identified. Each patient selected had a distinct peak on their SPEP due to daratumumab, which was easily distinguishable from their M-protein. The daratumumab peak was measured by capillary electrophoresis and subsequently quantified using Sebia Phoresis software. In a setting where patient history is available, review of sequential patient Hydrashift results allowed for the determination of how often Hydrashift was ordered and at what point during the course of a patient's treatment the IFE transitioned from a true positive to a false positive. A thorough analysis of our workflow was also performed with particular attention being given to IFE and SPEP comments and interpretations.

Results: Daratumumab therapy adds in the range of 0.02 - 0.20 g/dL to a patients M-Spike. This interference was typically evident at the first SPEP performed after the initiation of daratumumab therapy. Since patient history is typically available during SPEP/IFE review at our institution, the Hydrashift was most often used when an IgG-kappa M-protein overlaps with daratumumab on IFE. Based on the M-protein concentration of daratumumab observed on SPEP, the assay is most useful when the M-spike is small (~0.2-0.3 g/dL). When the patient's M-Spike is in this range, we will see the IFE begin to transition from a true positive to a false positive. In the workflow we have formulated, interpretive comments are included in IFE results to indicate that the M-protein, daratumumab, or both, are visible by Hydrashift. If both M-protein and daratumumab are present, we indicate that the M-spike quantitation may be overestimated due to the presence of the drug. We have also used the Hydrashift assay when patient history is not known, and a small IgG kappa band is observed on IFE.

Conclusion: The Hydrashift 2/4 Daratumumab assay provides a way to address the issue of false positive IFE results due to daratumumab interference. In the setting where patient history and historical IFE results are available, the assay is most useful for patients with IgG kappa M-proteins that overlap daratumumab, particularly when the patient's M-spike is small. Use of Hydrashift is typically not needed in patients with large M-spikes (>0.3 g/dL). Comments describing the composition of the M-spike allow the clinician to correctly classify the patient's disease status.

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Urine Sample Studies in the Follow-Up of Patients with Monoclonal Gammopathies: Is It Really Necessary?

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Background:

The high incidence and prevalence of monoclonal gammopathies represents a challenge for clinical laboratories where these patients are monitored. One of the cornerstones is the management of urine samples. Their study implies a high consumption of time, human resources and money. Moreover, urine techniques have limitations and recent studies have questioned the value of those. In this context and taken into consideration the IMWG criteria for measurable disease, we have established an algorithm through which we can decide when to give up on urinary tests without causing any harm in patients 'follow up or while making clinical decisions. In order to decrease the use of urine techniques we propose an algorithm including serum free light chain (sFLC), serum protein electrophoresis (SPE) and the patient's 24-hour proteinuria.

Methods:

All urine studies from 2018, from samples with a recognizable MP by SPE, were retrospectively evaluated. A total of 657 samples were included and their results for sFLC, SPE and 24-hour urine protein were compared to corresponding urine electrophoresis (UPE) and immunofixation (UIFE). We assumed that 24h-urine protein levels<200mg, and/or serum MP<1g/dL, and/or involved serum iFLC<100 mg/L in samples from these patients eliminate the need to perform urinary tests for MP measurement. Negative predictive values (NPV) were determined. Statistical analysis were carried out with GraphPad Prism 8 software.

Results:

344/657 (52.3%) samples had urinary protein level <0,2g/24h, of which 131 had measurable and 213 had unmeasurable serum disease according to SPE and iFLC. Only 3 (1.4%) out of the 213 samples with unmeasurable disease in serum and low urinary protein level had a positive UPE; all 3 patients had abnormal sFLC ratio that include 1 IgA MM, 1 waldenström macroglobulinemia and 1 biclonal gammopathy IgA+IgG, all of them with a comigration of the MP in the ß-region making it difficult to determine MP concentration. 48/213 (22.5%) showed a MP on UIFE, 11 of which with normal sFLC ratio. All considered, the algorithm designed shows a NPV of 99% and a sensitivity of 99% for measurable UPE; and a NPV of 77% and a sensitivity of 88% for positive UIFE. Although the lower NPV/sensitivity for UIFE, most of these samples did not have quantifiable MP by UPE. Reaching a NPV of 99%, almost all samples with a negative result in our algorithm will result in a negative UPE, reducing the need to perform the urine assay in these cases. The algorithm was prospectively introduced in our center, from June to December 2019. During this period, 952 UIFE were requested and, according to this model, 295 (31%) didn't meet the criteria to proceed with UPE/UIFE. As an overall estimated cost of 107,26 euros per UPE/UIFE, the hospital saved 31,565 euros in seven months.

Conclusion:

The proposed algorithm is shown to be helpful to make a more selective use of UPE and UIFE. No relevant clinical information is omitted in virtually any case in which the urine study is not performed.

B-359

Plasma EGFR T790M Detection Rate in NSCLC Patients using Droplet Digital PCR

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Background: Around 85% of lung cancer cases are non-small cell lung cancer (NSCLC), and mutations that lead to sensitivity or resistance to *EGFR*-target therapy are well described. The three most relevant mutations in *EGFR* are exon 19 deletions (DEL19) and L858R activating mutations with sensitivity to tyrosine-kinase inhibitors (TKIs), and T790M with resistance to first- and second-generation TKIs, but sensitivity to third generation TKIs. **Objectives:** Describe the overall frequency of the aforementioned mutations in plasma from NSCLC patients, focusing on T790M. **Methods:** 397 Brazilian patients with NSCLC were analyzed between April 2018 and January 2020. Patients were submitted to plasma collection, cell-free DNA (cfDNA) isolation and *EGFR* mutation analysis for DEL 19, L858R and T790M by droplet digital PCR (ddPCR). **Results:** From the 548 samples, 327 (59.7%) were wild-type for all the three mutations tested. 221 (40.3%) were positive for one or more mutations, being 93 (17.0%) positive for T790M. Analyzing only positive results (n=221), T790M frequency reached 42.1%. **Discussion:** The *EGFR*T 790M positivity rate observed in literature for FFPE samples is around 50-60% in patients previously diagnosed for NSCLC, with detectable sensitivity mutations. Our data showed a lower *EGFR* T790M positivity rate (17.0%) in plasma samples, which could be due to pre-analytical factors: sampling time, cancer stage and previous treatment contribute to insufficient or undetectable cfDNA, increasing the false-negative rate. When analyzing only positive results, the positivity rate increased to 42.1%, which is similar to the observed in other studies using plasma.

B-360

Accuracy Enhanced Rare Cell Detection Driven by the nCyte® System with an AI Based Algorithm

S. Thomas[1], R. Mohl[2], Y. Yu[1], J. Kearny[1], M. Hrovatic[1], J. Bos[1], K. Murphy[1], P. Bernard[2], J. Andrade[1]. *[1]Axon Dx, LLC, Earlysville, VA, [2]ARUP Laboratories, Salt Lake City, UT*

Background Today, oncologists treating cancer patients are faced with limited sets of diagnostic tools. Tissue biopsies are the current method used to give a comprehensive picture, however these invasive biopsies typically provide limited information and can take weeks to get a diagnostic result. The use of a clinical diagnostic test, such as a liquid biopsy, can provide real time disease information that would greatly benefit monitoring and responding to disease progression. Axon Dx's approach to cancer diagnostics was to develop an automated scanning microscope (**nCyte**® system), which can scan millions of cells, processed without enrichment and returning only images of those rare cell events of interest. The **nCyte**® system provides a unique solution for detecting low levels of rare cells in a clinical setting through the combination of high resolution multichannel optical imaging, proprietary fluorescent taggants and assays, and state-of-the-art rare cell AI segmentation. The unique, high quality optical properties of the **nCyte**® system allow rare cell detection to be characterized visually after being selected from our proprietary algorithm; ultimately, providing physicians with information that could lead to more rapid detection of minimal residual disease and potentially reduce the number of invasive biopsies. **Methods** From a single blood draw, white blood cells and rare cells are isolated through a non-enrichment method, processed and labeled with our proprietary cocktail. The performance of the **nCyte**® system was evaluated using internal experiments for linearity/recovery and instrument-to-instrument variation. Axon Dx also performed a study partnering with ARUP to compare our system with that of the FDA cleared, CellSearch® system. Blood samples from 26 prostate cancer patients were collected by ARUP and analyzed using CellSearch technology. The remaining de-identified blood was shipped to Axon Dx for processing and analysis. **Results** Analysis using the **nCyte®** software (nAble™) demonstrated an ≥85% overall recovery, linearity between 1-300 target cells with an R^2 value of 0.98 in spiked blood samples. The data from CellSearch and **nCyte**® systems were analyzed using a Bland-Altman plot, which shows that all but one sample fell in the limits of agreement between -25 and 29. The samples were separated into three groups: 0-5 CTCs, 6-20 CTCs, and 21-100 CTCs with the bias of these groups being 3.33, -1.67, and -10.25, respectively. The **nCyte**® system was able to provide higher quality images with greater morphology detail than the CellSearch system. **Conclusions** The Axon Dx **nCyte**® system demonstrates its reliability for capturing rare cells with minimal losses. This underlines the ability to detect rare cells in unenriched samples, with high efficiency. There was also a strong correlation between CellSearch system and the **nCyte**® system, where 24 of the 26 patients agreed within a 95% confidence interval, moreover the **nCyte**® system seemed able to identify a greater number of intact CTCs when the counts were <20 CTCs. The **nCyte**® system shows superior image quality, potentially leading to improved identification and accuracy in CTC detection. Overall, the **nCyte**® system demonstrates superior imaging for a sensitive, efficient and reliable assay for the detection of circulating rare cells.

Wednesday, December 16, 2020

Poster Session: 12:30 PM - 1:30 PM

Late Breaking Session

B-361

Laboratory Changes and the Relationship between TB, HIV, HbA1c and SARS CoV2 in South Africa

J. George[1], R. Hesse[1], D. van derWesthuizen[2]. *[1]University of Witwatersrand, Johannesburg, South Africa, [2]University of Cape Town, Cape Town, South Africa*

COVID-19 related laboratory analyte changes and the relationship between SARS-CoV-2 and HIV, TB and HbA1c in South Africa

The aim of this study was to describe the biochemical and haematological analyte changes seen in COVID-19 patients using South African laboratory data, and to determine the effect of HIV, TB and DM on the risk for acquiring SARS-CoV-2 and the outcomes as measured by intensive and high care admission.MethodsThis was a retrospective analysis of all data for individuals that had at least one PCR test for SARS-CoV-2 at any of our laboratories from the period 1st March to 7th July 2020. Test results for TB, HIV and HbA1c was taken from the six months prior to SARS-CoV-2 testing. Outcome data was not available so we used ICU/high care or critical care admission to determine disease severity. We reported prevalence of HIV and TB for critical versus non-critical groups. HbA1c was stratified into one of 4 categories: [1] optimal control and normal (<5.7%); [2] controlled or pre-diabetic (5.7 – 6.49%); [3] uncontrolled diabetic (6.5 – 10%); [4] poorly controlled diabetic (>10%). CD4 counts were categorized into one of eleven bins, with increments of 100 cells/uL between bins, ranging from 0-99 cells/uL (bin 1) to ≥1000 cells/uL (bin 11). The statistical significance between groups for all results was calculated by Wilcoxon rank-sum test for non-parametric data, the Student's t-test was used for parametric data and Pearson's Chi-square test was used for proportions. A p-value of <0.05 was regarded as significant.ResultsWe report data for 842,197 individuals, of which 11.7% (98,335) had at least one positive SARS-CoV-2 PCR test, and 88.3% (743,862) tested negative. The mean age for the positive group was 42.3±15.0 years vs. 42.6±14.7 years in the negative group and female prevalence was 61.6% (60,545) vs. 56.3% (419,011) (p<0.001 for both), respectively. The overall prevalence of HIV was 6.3% and did not differ between positive and negative groups, but was higher in the critical group (9.15%) than in the non-critical group (6.24%) (p=0.011). The prevalence of uncontrolled diabetes was 3.4 times higher in SARS-CoV-2 positive cases (p<0.001) but was not higher in the critical vs. non-critical cases (p=0.612). The prevalence of TB in SARS-CoV-2 negative individuals was higher than in the SARS-CoV-2 positive group (p<0.001). The neutrophil-to-lymphocyte-ratio, coagulation markers, urea, cardiac, and liver related analytes were significantly elevated in the critical compared to non-critical cases. Platelet count and creatinine concentration did not differ significantly between the two groups.ConclusionsOur findings did not support an increased prevalence of either HIV or TB in individuals with SARS-CoV-2 infection but did indicate an increase in disease severity with HIV-positive status. Our findings of clear differences in several commonly measured analytes between the critical and non-critical group suggest that these may be useful in our setting to triage patients.

B-362

Critical Role of Hematology Laboratory Parameters in Severe COVID 19 Infection: A Case Report from Orbassano, Italy

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Background and Purpose COVID 19, as a systemic infection, has prominent impact on the hemopoietic system. Obesity as a risk factor in various diseases, is also linked with COVID 19 severity. Here we present for the first-time dynamics of hematology parameters in severe COVID-19 patient who was a known case of beta thalassemia carrier with risk factor of severe obesity (BMI=54kg/m^2). **Case Description and Methodology** A 45-year-old female presented to ED with COVID-19 symptoms and later confirmed with a Positive viral PCR. Disease got severe with complications of intubation, coagulopathy, septic shock with renal insufficiency, with anemia requiring transfusion therapy. CBC+Diff tests were performed using the Abbott Alinity hq hematology analyzer as part of routine laboratory monitoring. This analyzer uses an advanced version of the Multi Angle Polarized Scatter Separation (MAPSS™) technology and reports a six-part White Blood Cell (WBC) differential, including Immature Granulocytes (IG). Patient was recovered and discharged after 14 weeks of hospitalization. **Results**

• During initial period of ICU stay, Total Leukocyte count increased with predominant Neutrophils. Peripheral blood smear examination during the septicemic episodes, reveals immature myeloid cells, with morphological changes in neutrophils. Alinity hq reported increasing concentration of IG, which was confirmed by peripheral blood smear examination. Lymphopenia at presentation was corelating with severity.

• During Recovery, Total Leukocyte Count, Neutrophils along with Immature Granulocytes (IG) showed progressive decline and lymphocytes also recovered to lower limits of normal reference range.

• Platelets initially showed inflammatory recruitment, followed by thrombocytopenia during consumption coagulopathy.

• Red cell cytograms, displaying red cell hemoglobin content and volume assisted in monitoring changes related to the developing anemia and subsequent transfusions. **Conclusion** The dynamic profile of Hematology parameters correlated with disease progression and assisted in predicting severity and therapeutic management in this severe COVID 19 case. High IG concentration (up to 8%) reported in a six-part Differential Complete Blood Count Test was coinciding with septic episodes.

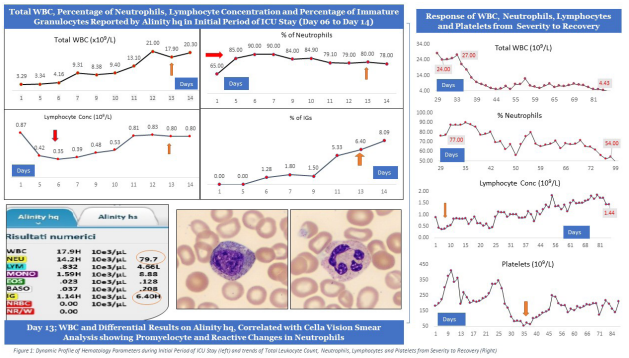

Figure 1: Dynamic Profile of Hematology Parameters during Initial Period of ICU Stay (left) and trends of Total Leukocyte Count, Neutrophils, Lymphocytes and Platelets from Severity to Recovery (Right)

B-363

Plasma Calprotectin Levels are Increased in COVID-19 Patients Admitted to ICU and Correlate with Endothelial Cell Damage

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Background: Corona virus disease 2019 (COVID-19) is a novel, viral-induced (SARS-CoV-2) potentially severe infection. Cytokine storm, increased neutrophil count and infiltration of the neutrophils in the lungs are observed in patients with severe COVID-19 disease. It is, however, not known to what extent neutrophil activation is related to COVID-19 severity and prognosis. Calprotectin is a major protein in the cytosol of neutrophils and is rapidly released upon neutrophil activation. The aim of the present study was to evaluate performance of calprotectin in prediction of disease severity in ICU-treated COVID-19 patients.

Methods: Twenty-seven patients admitted for COVID-19 infections to the intensive care unit (ICU) at Uppsala University Hospital were included in this observational study together with ten controls, post-operative cancer patients treated at the same ICU. Analysis of calprotectin was performed in plasma with particle enhanced turbidimetric assay (Gentian AS, Moss, Norway) and analysis of e-selectin was performed by ELISA (DY724, R&D Systems, Minneapolis, MN, USA).

Results: The COVID-19 patients had significantly higher calprotectin levels compared with the control group of patients admitted to the same ICU. Calprotectin levels differentiated between COVID-19 and non-COVID-19 patients with an area under

the curve of 0.996 according to the ROC analysis. Positive correlation was observed between levels of calprotectin and e-selectin, a biomarker for endothelial cell damage (Spearman R=0,455, p=0.006), indicating a potential role of calprotectin in prediction of damage of endothelial cells and subsequent organ failure.

Conclusion: Concentration of calprotectin in plasma is significantly elevated in ICU-treated COVID-19 patients, suggesting involvement of neutrophils and presence of an inflammatory cascade. As the release of calprotectin from neutrophils is very rapid in response to infections, calprotectin may be used as an early marker for neutrophil activation in COVID-19 infections. Moreover, calprotectin could be used as a predictor of endothelial cell damage and organ failure. Further studies are planned to confirm these findings.

B-364

Performance of the Roche Electrochemiluminescent IL6 Assay in SARS-CoV-2 Patients

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Introduction: IL-6 may serve as an early indicator of inflammation, and high levels of IL-6 have been observed in patients with severe COVID-19 infection. We report the performance of the Roche Elecsys IL-6 assay on the Cobas e801 immunoassay analyser.

Methods: The Roche IL-6 immunoassay is an electrochemiluminescent sandwich immunoassay, with a claimed measuring range of 1.5-5000pg/mL, and limit of quantitation (LOQ) of 2.5pg/mL. Using anonymised leftover sera, we assessed the assay linearity, analytical precision, LOQ and throughput as per CLSI protocols. We also compared the IL6 values of 17 SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) positive patients to their existing C-reactive protein (CRP)/procalcitonin (PCT) results. We employed a RT-PCR test system that targeted at least 2 viral epitopes of SARS-CoV-2. Statistical analyses were performed using MedCalc software version 19.4.0 (MedCalc Software Ltd, Ostend, Belgium).

Results: The IL-6 assay was linear from 1.60 to 4948pg/mL (slope 1.002, intercept -1.385). Inter-assay precision (CV) was good - 2.3% at 34.1pg/mL and 2.5% at 222pg/mL. Even at concentrations close to the lower limit of the measuring range (1.59pg/mL), CV was still 4.9% (95% CI 2.5-7.3%). Assay time is 18min and results are available 1 minute later; throughput for the analysis of 50 samples was 29 minutes. The correlation between IL6/CRP/PCT was good in patients who were SARS-CoV-2 RT-PCR positive [r = 0.73 (CRP) and 0.89 (PCT)]. When the 17 subjects were grouped based on initial CRP/PCT levels (both normal, CRP high, CRP and PCT high), the IL6 also showed a stepwise increase with CRP/PCT (see Table 1). Notably, in 5 out of 7 patients with normal CRP/PCT, the IL6 was increased (>7.00pg/mL).

Conclusion: The Roche IL-6 assay performance is good, within manufacturer's claims and fit for operational use. In SARS-CoV-2 RT-PCR positive patients, CRP/PCT closely correlates with IL6. IL6 precedes the rise of CRP/PCT.

TABLE 1: Relationship of IL6 with CRP and PCT in SARS-CoV-2 positive patients.

Group (N)	Mean IL6 (pg/mL) (min/max)	Mean CRP (mg/L) (min/max)	Mean PCT (ug/L) (min/max)
Normal CRP + PCT (7)	13.1 (2.55/35.2)	4.4 (<0.6/6.5)	0.17 (<0.06/0.22)
High CRP, normal PCT (8)	45.2 (2.18/152)	75.3 (19.3/139.0)	0.28 (<0.06/0.48)
High CRP + PCT (2)	673 (376/969)	165.6 (134.8/196.4)	8.06 (7.84/8.28)

(Abbreviations: CRP: C-reactive protein, PCT: procalcitonin)

B-365

Circulating Levels Calprotectin for Prediction of Disease Severity in Hospitalized COVID-19 Patients

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Background: In severe cases, the interaction of SARS-CoV-2 with the immune system contributes to a dysfunctional immune response, which triggers a cytokine storm that mediates widespread inflammation and multi-organ damage, major cause of disease severity in infected patients. Elevated blood levels of some biomarkers have been identified as outcome-related predictors, including inflammatory markers as C-reactive protein (CRP), ferritin and D-dimer. However, the potential role of emergent inflammatory biomarkers, such as calprotectin is less known. We examined the value of serum calprotectin for prediction of in-hospital mortality and need of mechanical ventilation (MV) in hospitalized COVID-19 patients. **Methods:** This was a prospective, observational study in hospitalized patients with confirmed SARS-CoV-2 infection. A blood sample for analysis was collected at Emergency Room, including CRP, ferritin, D-dimer and calprotectin. Serum calprotectin levels were measured by a particle enhanced turbidimetric immunoassay (PETIA) (GentianAS, Norway) on a Cobas c 502 instrument (Roche Diagnostics, Mannheim, Germany). The discrimination ability to predict both outcomes was evaluated by Receiver Operating Characteristic (ROC) curve analysis. A binary non-adjusted logistic regression model was generated for the prediction of in-hospital mortality, according to the optimal threshold for each biomarker maximizing the Youden index. **Results:** The study population included 66 COVID-19 patients [mean age: 61 (SD: 16), male: 43 (65.2%)]. Main comorbidities were hypertension (47%) and diabetes mellitus (27.3%). Mortality rate in the cohort was 12.1% (8/66) and 9 (13.6%) patients required MV. For in-hospital mortality, CRP (19.3 mg/dL (95%CI: 10.4-30.5) vs. 6.0 (2.8-10.8); p=0.008), calprotectin (7.1 mg/L (4.5-10.3) vs. 3.1 (1.9-4.4); p=0.005) and D-dimer (3465 ng/mL FEU (995-4432) vs. 570 (404-848; p=0.001) levels were significantly higher in patientes who died; this difference was not found for ferritin (769 ng/mL (501-1301) vs 360 (223-1256); p=0,08). Calprotectin showed a good discrimination capacity (ROC AUC: 0.801, 95% CI: 0.691-0.894; p=0.001), similar to both D-dimer (ROC AUC: 0.869, 95%CI: 0.763-0.939; p<0.001) and CRP (ROC AUC: 0.791, 95%CI: 0.673-0.881; p=0.003). A calprotectin level ≥ 3.9 mg/L showed an unadjusted odd ratio of 13.30 (95%CI: 1.53-116; p=0.004). For need of MV, ferritin (1201 ng/mL (643-1526) vs. 362 (223-872); p=0.036), CRP (10.1 mg/dL (7.5-29.7) vs. 6.0 (2.8-10.9); p=0.048) and calprotectin (5.0 mg/L (3.6-8.4) vs. 3.2 (1.8-4.8); p=0.032) levels were significantly higher in patients requiring MV, without differences in D-dimer (630 ng/mL FEU (404-1044) vs. 600 (457-897); p=0.695) levels. ROC AUC were 0.723 (95%CI: 0.599-0.826; p=0.027) for calprotectin, 0.719 (95%CI: 0.595-0.823; p=0.031) for ferritin and 0.707 (95%CI: 0.582-0.812; p=0.044) for CRP, respectively. No patient with a calprotectin level < 2.9 mg/L, corresponding to Index Youden cutoff. **Conclusion:** In our study, only CRP and calprotectin showed a significant ability to predict both outcomes. Our findings suggest that calprotectin might have a potential role in the assessment of prognosis in COVID-19 patients. As one of the earliest biomarkers for neutrophil activaiton, calprotectin is of special interest for early identificaiton of patients at the risk for development of severe events and mortality. Further investigations are required to confirm our preliminary findings.

B-366

Use of Real Time IP-10 Measurements for Identification and Monitoring of the Dysregulated Immune Response in COVID-19 Patients

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Background: About 10% of COVID-19 patients progress to the most severe stage of illness, manifesting as an extra-pulmonary systemic hyperinflammatory syndrome. Corticosteroid treatment at this phase has been shown to reduce mortality. The non-specific inflammatory biomarkers CRP and Ferritin are employed to detect and assess

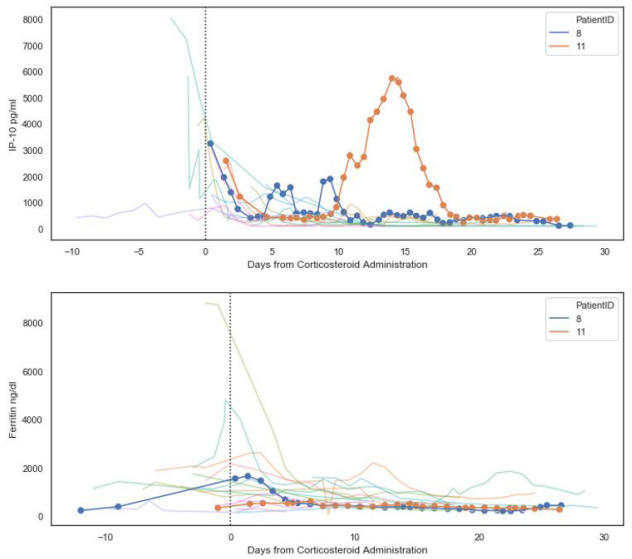
the inflammatory state of COVID-19 patients. Interferon gamma-induced protein 10 (IP-10) is an inflammatory marker that plays a role in the dysregulated host response of COVID-19 patients. MeMed Key™ is a rapid immunoassay platform that provides IP-10 measurements in 15 minutes. We hypothesized that providing physicians with IP-10 measurements would enable them to identify patients with a dysregulated immune response, potentially improving patient outcome.

Methods: From 7th April 2020 to 10th May 2020 serum remnants from routine blood draws were collected serially from 52 SARS-CoV-2 positive patients hospitalized at a COVID-19 dedicated medical center. A clinical decision support protocol was in place focused on managing oxygenation, inflammation and viral clearance. (NCT04389645).

Results: Among the 52 patients measured, 26 patients were classified as severe based on the COVID-19 severity score. Severe patients exhibited higher median IP-10 levels, 1190 pg/ml as compared to 328 pg/ml in the non-severe group ($p<0.01$), and higher median Ferritin levels, 652 ng/dl versus 283 ng/dl ($p<0.01$). Seventeen of the 26 severe COVID-19 patients were treated with corticosteroids. All patients exhibited reduction in IP-10 within 3-5 days of corticosteroid initiation; median IP-10 levels decreased from 2961 pg/ml to 372 pg/ml ($p<0.01$). Median Ferritin levels remained elevated, despite a slight decrease from 821 ng/dl to 610 ng/dl. Patients #8 and #11 were the only two patients that exhibited IP-10 flare-ups >1000pg/ml and eventually died of COVID-19 related complications.

Conclusion: Real time IP-10 measurements help identify COVID-19 patients in a hyperinflammatory state, specifically those not responding to standard corticosteroid regimens, who may require more aggressive treatment. Further studies are warranted.

Figure 1: Initiation of corticosteroid therapy was reflected by a decrease in IP-10 and Ferritin levels in severe patients treated with corticosteroids (n = 17)



B-367

A Unique Rapid Test to Determine Neutralizing Antibodies Directed against SARS-CoV-2

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Background: The SARS-CoV-2 epidemic started in December 2019 and became a world-wide pandemic that is still ongoing with major impacts not only on health, but also on the daily life of everyone.

Objective: In order to assess neutralizing antibodies directed against the novel coronavirus SARS-CoV-2, our intention was to develop a serological rapid test which is clearly different from most other rapid tests available so far. Thus, the BÜHLMANN Quantum Blue® SARS-CoV-2 RBD^{plus} Lateral-Flow assay was designed to result in a qualitative test with high specificity and sensitivity due to the simultaneous detection of antibodies of various isotypes (including IgM, IgA, IgG) directed against the SARS-CoV-2 Spike RBD domain.

Methods: Two recombinant antigens, Spike RBD^{plus} and a synthetic binder based on the nanobody scaffold specifically recognizing the SARS-CoV-2 Spike RBD were developed. The novel RBD^{plus} antigen is used as labelled antigen as well as antigen on the test line, whereas the synthetic binder is used for the control line. With this unique assay design, sera of 30 RT-PCR positive Covid-19 patients, that were collected at least 21 days after diagnosis, and 100 negative sera were analyzed. The signal on the test line was evaluated by eye after 15 minutes, but also with the BÜHLMANN Quantum Blue® reader to get more objective results.

Results: Our recombinant RBD^{plus} antigen is a highly thermostable, extended RBD that, unlike canonical RBD constructs, does not form disulfide-bridged dimer artefacts in solution. This antigen is very well suited to capture SARS-CoV-2 RBD antibodies on the test line. In this set-up, bi- or multivalent antibodies that are specific to the SARS-CoV-2 RBD efficiently bridge the antigen on the T-line with the same antigen conjugated to nanoparticles irrespective of their isotype and species. A specificity of 99% and sensitivity of 93.3% was achieved with this novel test format. Patient samples with negative results in our set-up using the Spike RBD^{plus} (no test line visible) were subsequently measured with the EUROIMMUN Anti-SARS-CoV-2 ELISA IgG, which uses a Spike construct as antigen, and the Epitope EDI™ Novel Coronavirus COVID-19 ELISA that recognizes antibodies directed against the Nucleocapsid of the novel coronavirus. According to the ELISA results those samples showing a negative result in our rapid assay appear to contain anti-SARS-CoV-2 antibodies mainly directed against the Nucleocapsid. Hence, the "real" sensitivity of our test to detect neutralizing antibodies in previously Covid-19 infected patients is well above 95%. Human serum as well as plasma and capillary blood was successfully analyzed for antibody reactivity.

Conclusion: The BÜHLMANN Quantum Blue® SARS-CoV-2 RBD^{plus} Lateral-Flow assay recognizes neutralizing anti-RBD antibodies with high sensitivity and specificity and will be a valuable diagnostic tool for efficient healthcare management during the Covid-19 pandemic. The antibodies that are recognized by this rapid test generally interfere with ACE2 receptor binding of the Spike protein and therefore with cell entry of the virus. Thus, our rapid test correlates with neutralizing antibody titers of patients formerly infected with Covid-19 and is likely to serve as diagnostic tool to predict their current immune status and susceptibility to another infection.

B-368

Performance Evaluation of Abbott SARS-CoV-2 IgG and IgM Assays

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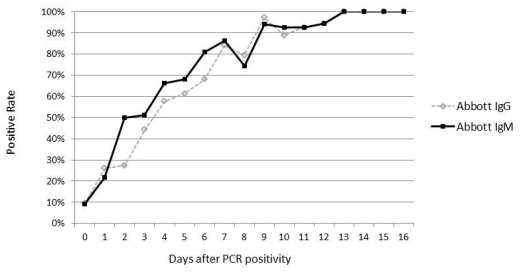
Background: Serological testing has recently emerged as an option to assist with determining exposure to SARS-CoV-2, the causative agent of COVID-19. There have been limited reports in the US that describe the kinetics and possible utility of tests that measure IgM and IgG to the novel coronavirus. The Abbott Architect SARS-CoV-2 IgG and IgM assays detect the presence of serum IgG to the SARS-CoV-2 virus nucleocapsid protein and IgM to the spike protein, respectively. This study evaluated the performance of these assays on the serum specimens of patients who were previously tested for the presence of the SARS-CoV-2 virus by reverse transcriptase-polymerase chain reaction (RT-PCR). **Methods:** Sensitivity specimens (n=492) were derived from excess serum sent for clinical testing from patients who tested positive for SARS-CoV-2 by RT-PCR during March and April 2020. The samples were drawn between day 0 and day 16 post molecular testing. Specificity samples (n=315) were derived from excess serum from patients who tested negative by RT-PCR during the same time period. The manufacturer's recommended index value cutoff of 1.40 was used for SARS-CoV-2 IgG and the cutoff of 1.00 for IgM. This work was approved by the Beaumont Research Institute (IRB#2020-233). **Results:** The sensitivity of the SARS-CoV-2 IgG and IgM assays from the date of PCR positivity are shown in Figure 1. The sensitivity was: 27.5% (11/40) for IgG and 50.0% (20/40) for IgM at day 2, 84.0% (42/50) for IgG and 86.3% (44/51) for IgM at day 7, 100% for both IgG and IgM beyond day 13. The specificity was 97.8 % (308/315) for both SARS-CoV-2 IgG and IgM assays. **Conclusion:** Our data demonstrate excellent analytical performance of the Abbott SARS-CoV-2 IgG and IgM assays. During the first week after PCR positivity, the Abbott SARS-CoV-2 IgM assay showed an improved sensitivity compared with IgG assay.

Figure 1: Clinical sensitivity of Abbott SARS-CoV-2 IgG and IgM assays.



B-369

A Case Report of Abnormal Seroconversion of IgG and IgM Occurred in Asymptomatic SARS-CoV-2 Infection

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Background: Nucleic acid test may lead to false negative results due to several factors in the diagnosis of COVID-19. We reported a case of IgM-positive but IgG and RT-PCR negative, and finally confirmed SARS-CoV-2 infection. **Case Presentation:** A 22-year-old male with no symptom of fever and cough, but with a history of contacting with his cousin in Chongqing, who came back from Wuhan, presented to our hospital to screen COVID-19 for resuming work on Feb 27. Antibody detection for COVID-19 was tested at first using MCLIA kits (Bioscience) showed IgM titer was 11.43 S/CO and IgG was 0.38 S/CO. Nucleic acid tests of nasal, pharyngeal and anal swabs on admission were negative. Laboratory results included blood routine, coagulation function, liver enzyme levels and a total bilirubin level and chest CT were all normal. Then further periodic follow-up was conducted, the titer of IgM gradually increased, peaked at 29.32 S/CO on Mar 9, then gradually began to decline, and reached the lowest value 5.97 S/CO on Mar 25, with the titer of IgG and RT-PCR tests were continuously negative. Non-specific antibody adsorbent kits and continuous dilution experiment eliminated the interference of nonspecific antibodies. Testing multiple manufacturers of reagents, IgM epitope analysis by MCLIA and colloidal gold and denaturation experiment of 2-mercaptoethanol proved that the positive strip is SARS-CoV-2 IgM. Finally, using a pseudovirus-based neutralization assay, we think this person does have specific neutralizing antibodies of SARS-CoV-2. **Conclusion:** We appeal to the complementary and collaboration of different diagnostic tests for SARS-CoV-2, which need to be highly important to protect public health.

Table. The results of RT-PCR and serological test of SARS-CoV-2 during the whole period of observation.

Time	Serological test		Nucleic acid test		
	IgG	IgM	Nasal swab	Pharyngeal swab	Anal swab
Feb 27	0.38	11.43	negative	negative	negative
Feb 29	0.39	14.67	negative	negative	negative
Mar 1	0.34	15.64	negative	negative	negative
Mar 3	0.41	16.59	negative	negative	negative
Mar 5	0.30	24.94	negative	negative	negative
Mar 9	0.12	29.32	negative	negative	negative
Mar 13	0.33	11.86	negative	negative	negative
Mar 17	0.37	9.52	negative	negative	negative
Mar 21	0.18	9.83	negative	negative	negative
Mar 25	0.43	5.97	negative	negative	negative

Note: S: OD value of the sample; CO: cutoff value. All S/CO values > 1 are judged to be positive. Cutoff value is set to 90,000 in whole serological test.

B-370

Validation of Roche Immuno-Assay for Severe Acute Respiratory Virus 2 (SARS-CoV-2/COVID-19) in South Africa

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Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antibody testing is an important ancillary diagnostic to the reverse transcriptase polymerase chain reaction (RT-PCR) test and can detect prior infection for epidemiological purposes. We evaluated the Roche Elecsys™ chemiluminescent immunoassay (Geneva, Switzerland) at an academic laboratory in Johannesburg, South Africa at various time points post-infection. This assay detects antibodies against the SARS-CoV-2 nucleocapsid antigen.

Methods: Serum samples were collected from 312 convalescent donors (with confirmed positive SARS-CoV2 PCR tests) with approval from the University of the Witwatersrand human research ethics committee (M200694). Samples were stratified by number of days post-diagnosis and symptoms (table 1). Samples stored prior to December 2019, including individuals with polyspecific antibodies, and asymptomatic patients with confirmed negative tests, were utilised as negative controls (n=131). All samples were stored at -80°C and analysed on a Roche cobas™ 602. We compared sensitivity and specificity of the Roche assay with RT-PCR and an in-house ELISA at various timepoints and in relation to severity of symptoms. Calculations were made utilising MedCalc statistical software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium).

Results: A summary of the sensitivities is included in Table 1. A cumulative specificity was 92.4% (CI 89.30% - 97.82%). 14 PCR-positive participants tested negative on both the Roche and the in-house ELISA suggesting a possible undetectable antibody titre.

Conclusion: SARS-CoV2 serology is an important ancillary diagnostic. Our evaluation revealed a sensitivity of 88% in patients at approximately 31 days post-diagnosis and that sensitivity was increased in symptomatic patients. Antibody responses waned over time with a corresponding reduced sensitivity. The sensitivity compared with PCR was lower than reported in some studies. This may reflect an absence of antibodies in some patients (including asymptomatic individuals). We conclude that this test shows utility in certain subsets of infected patients.

Table 1: Sensitivities calculated by days post-diagnosis and symptoms

Sensitivities post diagnosis			
Days post PCR diagnosis	**Number of positive patients analysed**	**Sensitivity**	**Confidence interval**
Overall	312	65.16%	59.57%-70.46%
Days 0-7	63	52.38%	39.41%-65.12%
Days 8-14	57	54.39%	40.66%-67.64%
Days 15-21	43	72.09%	56.33%-84.67%
Days 22-30	44	47.73%	32.46%-63.31%
Days 31-50	44	88.64%	75.44%-96.21%
>50 days	59	79.66%	67.17%-89.02%
Sensitivities according to symptoms			
Asymptomatic	33	57.58%	39.22%-74.52%
Mild disease (upper respiratory tract symptoms only)	49	59.18%	44.21%-73.00%
Moderate disease (systemic symptoms)	90	64.44%	53.65%-74.26%
Severe disease (requiring admission)	63	69.84%	56.98%-80.77%

B-371

Detection and Quantification of SARS-CoV-2 RNA by Digital Droplet PCR <ddPCR> Assay

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Background: Severe acute respiratory syndrome coronavirus 2 <SARS-CoV-2> is a positive-sense, single-stranded RNA virus that causes coronavirus disease 2019 <COVID-19> involving both upper and lower respiratory tracts. Symptoms can range from mild <i.e., the common cold> to severe <i.e., pneumonia> in both healthy and immunocompromised patients. SARS-CoV-2 is likely to be at the highest concentrations in the nasopharynx during the first 3 to 5 days of symptomatic illness. As the disease progresses, the viral load tends to decrease in the upper respiratory tract, at which point lower respiratory tract specimens <eg, sputum, tracheal aspirate, bronchoalveolar fluid> would be more likely to have detectable SARS-CoV-2.

Objective: To assess the analytical performance of a ddPCR assay for detection and quantification of SARS-CoV-2 RNA in clinical nasopharyngeal <NP> and oropharyngeal <OP> swab specimens.

Methods: We used heat inactivated, cell line-derived SARS-CoV-2 virion <ATTC® VR1986TMHK> and appropriate assay controls <EXACT Diagnostics, BioRad Laboratories, Hercules, CA> to test clinical NP and OP swabs collected from symptomatic patients and placed in viral transport media or PBS solution. Total nucleic acid <TNA> extraction and purification was performed using easyMAG instrument system <bioMérieux Inc., Durham, NC>. Simultaneous quantification of the SARS-CoV-2 nucleocapsid <N1 and N2> and human RNase P-encoding <RPP30> sequences was performed using AutoDG and QX200 ddPCR multi-well system <Bio-Rad Laboratories>. We assessed sample stability, limit of detection <LoD>, upper and lower limits of quantitation <LoQ>, linearity, analytical precision, accuracy, and cross reactivity.

Results: SARS-CoV-2 viral load remained stable in transport media containing NP and OP swabs when tested up to 3 days at 15°-25°C, 10 days at 4°C, and 30 days at -70°C. LoD was established at 191 copies/mL of transport media based on 90% detection rate <95% CI; 140 - 336>, with the lower LoQ at 500 copies/mL based on 100% detection rate. Serial dilution of heat-inactivated SARS-CoV2 virions showed linear quantification from 100 to 10,000,000 copies/mL. Intra- and inter-assay variability of the assay was <0.5 $\log_{10}$ copies/mL in S.D. at various viral load levels. Total agreement of qualitative results <detected vs not detected> was observed in 42 unique known-positive and 21 known-negative clinical swab specimens previously tested with the Abbott RealTi*m*e SARS-CoV-2 assay <Abbott Molecular Inc., Des Plaines, IL>. After calibrating the Abbott assay with a 7-level panel prepared from the ATCC SARS-CoV-2 virions at 10^1 to 10^7 copies/mL, Deming regression analysis of the quantitative results generated from the Abbott and ddPCR assays on 20 known-positive clinical NP or OP swab specimens showed good correlation <R^2 of 0.9726 and 0.9713 for the N1 and N2 targets, respectively>. There was no cross-reactivity of the common seasonal coronavirus strains <229E, OC43, and NL63> with this ddPCR assay.

Conclusion: The quantitative SARS-CoV-2 assay by the ddPCR method is fast, reliable, and accurate in detecting and quantifying SARS-CoV-2 RNA in clinical NP and OP swab specimens, and it may be a useful tool in serial monitoring of viral load in the respiratory tracts of patients for evaluating the efficacy of therapy for COVID-19.

B-372

An Enrichment-Based Next-Generation Sequencing Workflow for Diagnosing and Characterizing SARS-CoV-2 and Other Respiratory Viruses

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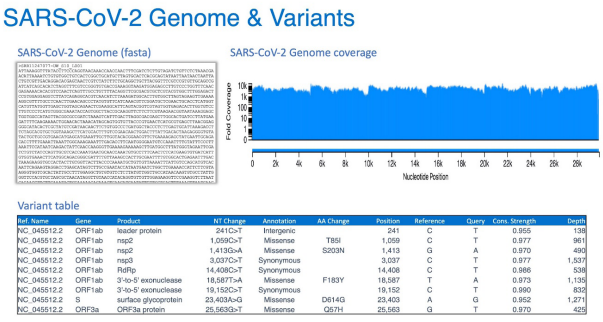
Background: The role of clinical metagenomics in routine diagnostics for infectious diseases is rapidly increasing. Here, we evaluate an enrichment-based NGS workflow to detect and characterize respiratory pathogens.

Materials: To determine the limit of detection (LoD), inactivated SARS-CoV-2 was serially diluted in simulated BAL fluid (5x10^3 human A549 cells/mL of universal transport medium) or a pool of residual sputum samples. Also, 12 SARS-CoV-2 PCR-positive and 12 PCR-negative residual nasopharyngeal swab (NP) samples were tested with the enrichment-based workflow. cDNA libraries were generated from extracted RNA using Illumina's TruSeq™ RNA Library Preparation Kit V2 and enriched using a respiratory virus oligo panel (RVOP, Illumina) before sequencing. Both pre-enriched and RVOP-enriched libraries were sequenced. Analyses were done using the Explify® RVOP analyses app (IDbyDNA) in BaseSpace. Sequencing data were down-sampled to 500,000 and 200,000 reads to determine the required sequencing depth.

Results: The LoD for SARS-CoV-2 was 1.2x10^2 copies/mL in simulated BAL samples and 1.3x10^3 copies/mL in sputum. SARS- CoV-2 was detected in 12/12 (100%) PCR-positive NP samples with enrichment-based sequencing but only in 8/12 (67%) pre-enriched libraries. Notably, SARS-CoV-2 was detected in 1/12 (8%) PCR-negative NP sample with the enrichment-based approach but not in pre-enriched libraries. Sequencing recovered complete SARS-CoV-2 genomes for viral typing (Figure). Furthermore, both sequencing approaches detected additional respiratory viruses in 6/12 (50%) SARS-CoV-2 PCR-negative samples, i.e., HRV-A (n=1), HRV-C (n=2), RSV B (n=1), influenza B virus (n=1), and HMPV (n=1). While results with 500,000 reads/sample reduced sensitivity in low positive detections, no further loss of sensitivity was observed with 200,000 reads/sample.

Conclusions: An enrichment-based NGS workflow for respiratory pathogen detection demonstrated high analytical sensitivity and reduced sequencing cost, allowing for higher throughput on existing platforms. Moreover, it enabled the detection of co-infections and SARS-CoV-2 typing, making it an essential tool for diagnostic and surveillance applications in pre- and post-vaccine scenarios.



Ref. Name	Gene	Product	NT Change	Annotation	AA Change	Position	Reference	Query	Cons. Strength	Depth
NC_045512.2	ORF1ab	leader protein	241C>T	Intergenic		241	C	T	0.955	138
NC_045512.2	ORF1ab	nsp2	1,059C>T	Missense	T85I	1,059	C	T	0.977	961
NC_045512.2	ORF1ab	nsp2	1,413G>A	Missense	S203N	1,413	G	A	0.970	490
NC_045512.2	ORF1ab	nsp3	3,037C>T	Synonymous		3,037	C	T	0.977	1,537
NC_045512.2	ORF1ab	RdRp	14,408C>T	Synonymous		14,408	C	T	0.986	538
NC_045512.2	ORF1ab	3'-to-5' exonuclease	18,587T>A	Missense	F183Y	18,587	T	A	0.973	1,139
NC_045512.2	ORF1ab	3'-to-5' exonuclease	19,152C>T	Synonymous		19,152	C	T	0.990	832
NC_045512.2	S	surface glycoprotein	23,403A>G	Missense	D614G	23,403	A	G	0.952	1,271
NC_045512.2	ORF3a	ORF3a protein	25,563G>T	Missense	Q57H	25,563	G	T	0.970	425

B-373

Comparison of 5 Serological Assays for the Qualitative Detection of SARS-CoV-2 Antibodies in a Healthy Adult Population

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Background: Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a newly discovered coronavirus (CoV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although much is unknown in terms of immunity or antibody formation for SARS CoV-2, dozens of immunoassays have gained EUA status by the FDA. While performance of these assays has been assessed by each manufacturer, very few studies have assessed these serological assays in a comparative fashion or across large populations of individuals that have a heightened risk of exposure to SARS CoV-2. This study assessed the performance of the following EUA serological assays for the qualitative detection of SARS-CoV-2 antibodies in a population of 200 healthy adults: Autobio anti SARS CoV-2 rapid test, Cellex qSARS-CoV-2 IgG/IgM rapid test, Diasorin LIAISON® SARS-CoV-2 IgG assay, Roche Elecsys® anti-SARS-CoV-2 assay, and Siemens SARS-CoV-2 Total assay.

Methods: Whole blood samples were collected from 200 subjects and processed. Serum samples from each subject were frozen at -70°C in at least 10 individual aliquots. Serum aliquots were analyzed by each of the assays of interest. All subjects with positive or reactive anti-SARS-CoV-2 antibody results for any of the 5 assays from Visit 1 had additional visits approximately 4 and 12 weeks after Visit 1. The study lasted approximately 16 weeks.

Results: When results were compared across all five assays, there was a significant difference in qualitative results of the 200 subject samples at Visit 1. In addition, significant differences were seen between the two rapid, lateral flow assays, but not between the high throughput immunoassays assessing total antibodies. Lastly, differences in agreement of positive qualitative results were noted between assays that evaluated IgG-only antibodies versus total antibodies to SARS-CoV-2.

Conclusion: The results of this study demonstrate inherent differences among serological assays for SARS-CoV-2 antibodies and the utility of each in screening large populations of individuals at heightened risk of infection.

B-374

Fluorescent Molecularly Imprinted Polymers (MIPs) for COVID-19 Detection

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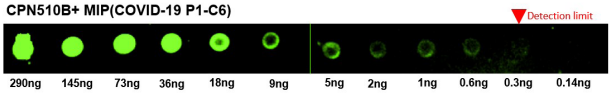
Background: MIP Diagnostics Limited produces nanoMIPs (nano-sized molecularly imprinted polymers) that are suitable for use in clinical diagnostic systems as a synthetic alternative to antibodies. They have unique properties compared to traditional binding molecules, not least the ability to be autoclaved and still retain functionality. Stream Bio Limited produces Conjugated Polymer Nanoparticles (CPNs™) that are highly fluorescent nanoparticles containing semiconductor light emitting polymer (LEP) cores encapsulated within a water friendly capping agent. With fluorescent properties significantly exceeding those of other molecular probes and labelling agents, CPNs™ offer immense brightness and sensitivity due to exceptional extinction coefficients and outstanding photo-, thermo- and chemical stability. Methods: nanoMIPs specific to a the spike protein of SARS-COV-2 (COVID-19) - were synthesized using proprietary methodology, whereby a peptide specific to a region of the whole spike glycoprotein molecule was immobilised on a solid phase, monomers and cross-linker were added, controlled polymerisation was initiated and, ultimately, nanoMIPs with high affinity for the spike protein were eluted. The affinity of the nanoMIPs for the spike glycoprotein was assessed using SPR (surface plasmon resonance). nanoMIPs were then conjugated to CPNs™ and evaluated using dot blot for spike glycoprotein detection. Results: SPR analysis returned a K_D of 15nM. Dot blot analysis returned a detection limit of under 0.3ng of spike glycoprotein using a simple optical set-up.



Conclusion: MIP Diagnostics proprietary nanoMIPs specific for SARS-COV-2 (COVID-19) tection of COVID-19 with a very long shelf-life under ambient conditions. The combined reagent provides assay developers with a new, significantly more robust, option for producing assays that could lead to a new generation of point of care and decentralised testing for COVID-19 with extended shelf life, no need for controlled shipping and storage and with significant enhancements in sensitivity.

B-375

Clinical Evaluation of the Truvian Easy Check COVID-19 IgM/IgG™ Lateral Flow Device

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Background: In the ongoing COVID-19 global pandemic, serological assays have become increasingly available through the FDA emergency use authorization (EUA). Although reverse transcription polymerase chain reaction (RT-qPCR) remains the gold standard testing for diagnosing active infection by SARS-CoV-2 via direct detection of viral genetic material, serological assays offer a distinct means to monitor and assess viral exposure history and response. Potential applications of serological testing include epidemiological surveillance for public health guidance, identification of plasma donors, and evaluation of vaccine response. However, at present, widespread application of serological assays has been limited in large part by reports of faulty or inaccurate tests. Therefore, rapid COVID-19 antibody tests need to be thoroughly validated prior to their implementation.

Methods: The *Easy Check IgM/IgG* device, developed against nucleocapsid and S1 spike protein RBD epitopes of SARS-CoV-2, was analytically evaluated and its clinical performance was analyzed with PCR-confirmed samples and a 2015 pre-pandemic reference sample set. The device performance was further characterized for cross-reactivity using sera obtained from patients infected by other viruses and for potential interference by biotin, bilirubin, hemoglobin, and triglycerides. Serial time-courses were also obtained with confirmed SARS-CoV-2-positive patient samples. The clinical performance was compared with the Roche Elecsys® anti-SARS-CoV-2 antibody assay.

Results: The *Easy Check* device showed excellent analytical performance and compares well with the Roche Elecsys antibody assay, with an overall concordance of 98.6%. Evaluation of the clinical performance of the device yielded a sensitivity of 96.6% and a specificity of 98.2% for samples derived from patients 14 or more days since initial PCR-positivity of SARS-CoV-2. Assuming a disease prevalence of 5%, the positive and negative predicative values are 74.0% and 99.8%, respectively, with an overall accuracy of 98.1% for this study population. The device performed robustly in the presence of biotin, bilirubin, hemoglobin, and triglycerides spiked into the samples at relatively high concentrations and no cross-reactivity was observed with samples obtained from patients infected with other respiratory virus strains nor with Hep B, Hep C, and HIV positive samples. A pilot study examining the use of fingerstick blood versus serum showed no observable difference when applied to the *Easy Check* device.

Conclusion: The *Easy Check* device is a simple, reliable, and rapid test for detection of SARS-CoV-2 seropositivity and its performance compares favorably against the well-established, automated Roche Elecsys antibody assay. This device has been approved via the FDA EUA for detection of exposure to SARS-CoV-2 infection.

B-376

Comparison of the Beckman Access and Abbott Architect COVID-19 IgG Serology Assay in a Cancer Population

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Background: Cancer patients are both immunosuppressed and undergoing chemotherapy, making them particularly susceptible to COVID-19. These homeostatic abnormalities could potentially lead to different antibody responses towards viral targets in COVID-19 IgG detection assays. Here we examined two IgG serology platforms, the Beckman Access 2, targeting the receptor binding domain of the spike (S) protein, and the Abbott Architect i2000, targeting the nucleocapsid (N) protein. The goal of this study was to determine which assay confirmed the presence of the COVID-19 IgG antibody at the earliest time point after the patient was confirmed to be COVID-19 positive by PCR.

Methods: Samples were received from patients receiving treatment at MSKCC for both cancer and COVID-19. Patient samples were saved (IRB approved) and organized by their number of weeks post PCR positive. A total of 88 samples over 4 weeks were collected encompassing 30 patients. Review of the medical record determined both cancer diagnosis and treatment regimen. Samples were analyzed for COVID-19 IgG reactivity on the Architect (Arc) and the Access (Acc). Assay results are reported in the terms of a calculated index value, where an index of ≥1.4 and ≥1.0 confirms IgG positivity on the Architect and Access respectively. The results of this study are represented as the percentage of IgG positive patients by week post PCR positivity.

Results: COVID-19 IgG positivity across both analyzers is as follows: week 0: Arc/Acc - 6.7%, week 1: Arc/Acc - 16.7%, week 2: Arc - 63.2% /Acc - 42.1%, week 3: Arc/Acc - 68.2%, week 4: Arc/Acc - 73.9%. 5 patients in the cohort did not test positive for COVID-19 IgG after 4 weeks elapsed. The sample cohort encompassed the following cancer diagnoses: lymphomas: 40%, multiple myeloma: 17%, breast cancer: 20%, other cancers: 23%. In this cohort, all but 3 patients were receiving chemotherapy, and of the 5 patients failed to develop an IgG response, 4 were receiving anti-CD-20 immunotherapy (Rituximab).

Conclusions: Both the Architect and Access platforms performed similarly at the week 0, 1, 3, 4 time points. Discrepancies in IgG positivity were found at the week 2 time point, where the Architect was able to detect the presence of the COVID-19 IgG in approximately 20% more of the patients as compared to the Access. IgG antibodies typically take upwards of two weeks to form suggesting that the Architect can detect COVID-19 IgG at lower levels than the Access. The discrepancy in IgG reactivity between the two analyzers suggests that the positive cutoff values for the Access may need to be adjusted. Lowering of the positive cutoff value could increase the test sensitivity with only a marginal effect on test specificity. Overall, IgG reactivity was found in 25/30 patients. Though our sample size is small, these results suggest that both analyzers will perform well when performing testing in either a cancer or immunocompromised patient population. 4/5 patients who failed to develop IgG antibodies had a recent history of Rituximab therapy, potentially impacting their immune response. Further studies are needed to confirm these findings.

B-377

Influence of Optimized Cut-Off Indexes on Two Automated Eletctrochemiluminescence SARS-CoV-2 Antibody Assays in the Early Detection of COVID-19

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Introduction: Reactivity for SARS-CoV-2 antibodies is defined by manufacturer's cut-off indexes (COIs) of ≥1.4 on the Abbott SARS-CoV-2 IgG (Architect i2000SR) assay and ≥1.0 on the Roche anti-SARS-CoV-2-Ab assay (CobasE801). We derived optimized COIs on both assays from the 99th percentile of healthy COI values and ROC analysis of healthy versus RT-PCR positive COIs, for the earlier detection of COVID-19.

Methods: Samples from RT-PCR positive patients (n=285, from 160 individuals) and healthy controls (n=980) were run on both assays. RT-PCR positive samples were stratified into 3 groups by days post-first positive RT-PCR (POS): 0-6 days (n=178), 7-13 days (n=47), and ≥14 days (n=60). We derived the 99th percentile COI values of the control population on each assay. ROC analysis was performed by comparing COIs of healthy controls and patient samples 0-6 days POS.

Results: The 99th percentile COI of control subjects were 0.17 (Roche) and 0.57 (Abbott) respectively. ROC analysis showed an associated COI criterion of 0.09 for the Roche (sensitivity 81.5%, specificity 90.7%) and 0.07 for the Abbott (sensitivity 77.0%, specificity 88.4%). Averaging the derived COIs from both approaches (99th percentile and ROC analysis) resulted in optimized COIs of 0.13 (Roche) and 0.32 (Abbott). Sensitivity of the Abbott/Roche assays increased stepwise with days POS. Applying these optimized COI limits, the sensitivity of both assays greatly improved: Abbott 46.1% to 60.1%, Roche 46.1% to 65.2% 0-6 days POS; Abbott 83.0% to 85.1%, Roche 80.9% to 89.4% 7-13 days POS; Abbott 98.3% to 100.0%, Roche 98.3% to 100.0% ≥14 days POS (see Table 1).

Conclusion: By applying optimized COIs for both the Roche (≥0.14) and Abbott (≥0.34), the sensitivities of both anti-SARS-CoV-2 assays improved substantially, especially in early COVID-19. This may facilitate the use of serology in early disease detection, in conjunction with RT-PCR or orthogonally.

TABLE 1: Assay sensitivities (positive percent agreement) - manufacturer versus optimized COIs

Days POS (N)	Abbott, COI ≥1.4		Abbott, COI ≥0.32		Roche, COI ≥1.0		Roche, COI ≥0.13	
	+ve / -ve	PPA% (95% CI)	+ve / -ve	PPA% (95% CI)	+ve / -ve	PPA% (95% CI)	+ve / -ve	PPA% (95% CI)
0-6 (178)	82/96	46.1 (38.6-53.7)	107/71	60.1 (52.5-67.4)	82/96	46.1 (38.6-53.7)	116/62	65.2 (57.7-72.1)
7-13 (47)	39/8	83.0 (69.2-92.4)	40/7	85.1 (71.7-93.8)	38/9	80.9 (66.7-90.9)	42/5	89.4 (76.9- 96.5)
≥7 (107)	98/9	91.6 (84.6-96.1)	100/7	93.5 (87.0-97.3)	97/10	90.7 (83.5-95.4)	102/5	95.3 (89.4-98.5)
≥14 (60)	59/1	98.3 (91.1-100.0)	60/0	100.0 (94.0-100.0)	59/1	98.3 (91.1-100.0)	60/0	100.0 (94.1-100.0)

(Abbreviations: COI: Cut-off index, POS: post positive RT-PCR, PPA: Positive percentage agreement, CI: Confidence interval)

B-378

Comparison of the Clinical Performance of Two Serological Tests for SARS-CoV-2: Rapid Test vs Chemiluminiscence Chilean Local Experience

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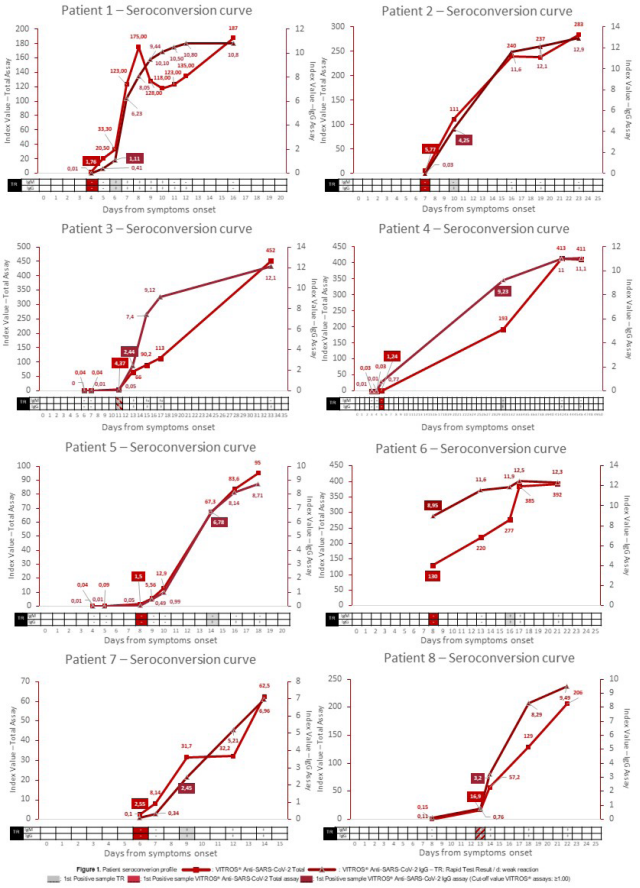
Background: Chemiluminescent assays for SARS-CoV-2 serology assessment are now available with high clinical sensitivity and specificity. The aim of this study was to show how testing technology impacts clinical characterization of COVID-19 patients. **Methods:** a total of 51 serum samples from 8 confirmed COVID-19 patients admitted in Clinica Redsalud Vitacura were tested for serology at different days from symptoms onset (DSO). Initial serology screening for SARS-CoV-2 was performed using rapid tests. Stored samples were reassessed using VITROS® Anti-SARS-CoV-2 Total and VITROS® Anti-SARS-CoV-2 IgG assays. We compared results from the rapid tests and VITROS® assays for each patient. **Results:** 100% clinical sensitivity was observed at > 8 DSO for the VITROS® Anti-SARS-CoV-2 Total assay (Fig.1), with earlier detection compared to rapid tests (6±8 days). From day 15, we still observed increasing amounts of antibodies, as per index values for total and IgG isotypes, but at a lower rate (Total: 13.9±5.4; IgG: 0.3±0.3 RU (Relative Units)/day), compared to initial DSOs (Total: 13.8±11.5; IgG: 0.8±0.6 RU/day). For one patient, rapid test showed a negative result in the last sample drawn 10 days after the first confirmed antibody positive draw (IgG-/IgM-), while VITROS assays remained reactive for sample series. **Conclusions:** VITROS® COVID-19 assays have shown usefulness to detect immune response with better performance compared to a rapid test. VITROS® Anti-SARS-CoV-2 Total assay which detects IgA, IgM and IgG antibodies, can detect immune response earlier and could be used to better assess immune response for patients having COVID-19. We were able to detect positive serology as early as 4 from DSO for one patient, versus 6 DSO for rapid test. Chemiluminescent assays should be used as the standard test for assessing serology for SARS-CoV-2 instead of rapid tests if the clinical performance of rapid tests is unknown or if its sensitivity and/or specificity are below current FDA standard.



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Analytical and Clinical Performance of Two Automated Anti-SARS-CoV-2 Immunoassays

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Background: The pandemic of coronavirus disease 2019 (COVID-19) is caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Antibody tests are important tools for monitoring and responding to the COVID-19 pandemic. Antibody tests with ideal reproducibility, sensitivity, and specificity should be used in public health and clinical settings. This study evaluated the analytical and clinical performance of the Abbott SARS-CoV-2 IgG and the Roche Anti-SARS-CoV-2 automated immunoassays.

Methods: Quality control samples, pooled COVID-19 and non-COVID-19 patient specimens were used for the reproducibility study. 246 residual specimens from 70 patients with COVID-19 diagnosis by RT-PCR were tested to study the sensitivity (positive percent agreement with COVID-19 diagnosis, PPA). 73 non-COVID-19 control specimens including 38 pre-pandemic samples, 30 samples from patients without COVID-19 diagnosis, and 5 samples from patients without COVID-19 diag-

nosis but with positive results of other respiratory pathogen infections were measured to study the specificity (negative percent agreement, NPA). All specimens were analyzed with both the Abbott and the Roche SARS-CoV-2 serological assays.

Results: The reproducibility of the Abbott assay was 100% (95% CI: 89% - 100%) for both negative and positive QC samples. For the Roche assay, the reproducibility was 100% (95% CI: 94% - 100%) for pooled COVID-19 negative samples, and 100% (95% CI: 89% - 100%) for pooled COVID-19 positive samples. The NPAs of the Abbott and the Roche assays were 100% (95% CI: 94% - 100%) and 97% (95% CI: 90% - 100%), respectively, but the difference between these two assays was not statistically significant (p = 0.500). The PPAs of the Abbott assay were 49% (95% CI: 41% - 56%) , 86% (95% CI: 74% - 93%) and 100% (95% CI: 76% - 100%) for the samples collected at 0-6 days, 7-13 days and ≥14 days after the first positive RT-PCR, while the PPAs of the Roche assay were 55% (95% CI: 47% - 62%), 86% (95% CI: 74% - 93%) and 100% (95% CI: 76% - 100%) for the same sample set. The difference for the specimens collected within 6 days after the first positive RT-PCR was statistically significant (p = 0.005). Among the 70 COVID-19 patients studied, 43 patients had daily specimen collection and their seroconversions were captured by both immunoassays. The median seroconversion days after the first positive RT-PCR test were 3 days for both the Abbott and the Roche assays. Within 7 days after the first positive RT-PCR test, 88% (38/43) (95% CI; 74% - 96%) and 91% (39/43) (95% CI; 77% - 97%) of patients became reactive by the Abbott and the Roche assays, respectively.

Conclusion: This study demonstrates similar analytical and clinical performance of the Abbott and the Roche SARS-CoV-2 antibody assays. The Roche assay is slightly more sensitive for patients in early stage of the disease. The specificity of the Abbott assay may be slightly higher than that of the Roche assay, but the difference is not statistically significant in this study.

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Antimicrobial Resistance Crisis after COVID-19

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Background After the COVID-19 pandemic, the world will struggle with a new dilemma of secondary bacterial or fungal infection, which may evolve to become resistant to antibiotics or anti-fungal. Antibiotic-resistant infections add considerable and avoidable costs to the already overburdened healthcare systems. The World Health Organization has been calling antimicrobial resistance (AMR) a major public health threat and it was estimated to kill around 700,000 people worldwide every year, a figure that was predicted to rise to 10 million by 2050. which drives us to find alternatives to these antibiotics especially natural ones. *Ricinus communis* is a soft wooden flowering perennial shrub, commonly known as castor. It was considered as the richest natural source of an antimicrobial peptide by application of its crude protein against three types of wide range antibiotic-resistant micro-organisms. **Objectives** Finding natural alternatives having a bacterial or fungal effect to reduce the indiscriminate use of antibiotics in cases of COVID-19. We tested the effect of total protein extracted from *Ricinus communus* by applying it on different bacterial and fungal isolates then, we determined the minimum inhibitory concentration (MIC). **Methods** First, we extracted protein from cereal seeds. Second, the test organisms were maintained on agar slant at 4 °C and subculture on a fresh agar plate. For disc diffusion and MIC assay, bacterial liquid cultures were initiated by placing a loop of bacteria from the slant into10 ml of LB media. Disc diffusion assay was conducted to detect the bacterial susceptibility to castor seed protein. The test organisms (100 µl) have been inoculated on the surface of the solid agar medium (Muller Hinton agar). The crude proteins from at the concentration of 50, 100, 200, and 400 µl/disc were impregnated on paper disc (6 µm). Then the agar plates containing microorganisms; soaked with paper discs were incubated at 37 ± 0.1 °C for 24 h. The protein concentrations that were provided inhibition zone of more than 10 mm were considered to be active and therefore their MIC determined. Antifungal activity of *Ricinus communis* seeds extracts was checked against fungal strains on Vogel Media. The MIC of a bacterium to a certain antimicrobial agent was determined by the micro-dilution method in a sterile flat bottom 96 well plate Standard (streptomycin, 1 mg/ml). The MIC is recorded as the lowest concentration of antimicrobial agent with no visible growth. **Results** Disc diffusion assay was conducted to detect the bacterial susceptibility to *Ricinus communis* against three different strains (*Staphylococcus aureus* MRSA ATCC 29213), (*Escherichia coli* ATCC 25922) and (*Candida albicans* ATCC 10231). MIC was 9.69, 4.84, and 77.5 in MRSA, E.COLI, and Candida respectively; when the number is large it means that is more resistant. **Conclusion** Before the SARS-CoV-2 pandemic, overcoming AMR already demanded urgent global action. Now it is even imperative to find an alternative to antibiotics. *Ricinus communis* seed protein has strong antibacterial and fungal activity.it also could help protect people against secondary bacterial infections that may be contributing to the significant levels of mortality seen with COVID-19.

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Longitudinal SARS-CoV-2 IgG Response Measured by Abbott Test in a Small Group of Healthcare Professionals

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Methods: When Abbott's SARS-CoV-2 IgG antibody test became available in May 2020, a pilot screening study was performed in the Department of Pathology of our hospital. On the voluntary basis, more than 95% of our employees participated in the project.

Results: Out of 125 tested individuals, 8 (6.4 %) were identified with positive results (signal to calibrator index (SCI) ≥ 1.4). Only 2 of those individuals were known to recover from COVID-19 (mild to moderate form). The remaining 6 participants had an exposure to sick individuals either in contact with close family member or in the hospital, without any significant symptoms. Although our IgG test is qualitative, the instrument reports the results in the aforementioned numeric index, which enables us to monitor this parameter over time. Seven of the initially positive employees were retested 2 months later. Their values remained elevated but had reduced numeric indices to 80% - 28% of the original value, with only one case of SCI below 1.4. 90% of all tested individuals had index results less than 0.16.

Conclusion: This small longitudinal project can add to the evaluation of natural history of the SARS-CoV-2 IgG response measured by the Abbott test.

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COVID-19 High Mortality Rate in Black and Duffy Antigen

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Background: As of August 4, 2020, the novel coronavirus disease 2019 (COVID-19) has been confirmed in 20,394,035 people worldwide with an approximately 3·53% mortality rate. COVID-19 infection is associated with an aggressive inflammatory response and release of a large amount of pro-inflammatory cytokines known as "cytokine storm." Cytokine storm is the release of interferons, interleukins, tumor necrosis factors, chemokines, and several other mediators. Chemokines are small protein molecules that act as chemoattractants, leading to the migration of immune cells to an infection site so they can target and destroy invading bodies such as microbes. The Duffy antigen is a broad receptor of chemokines in human blood. Early on, it was found that the overwhelming majority of people of African descent had the erythrocyte phenotype Fy(a-b-) (henceforth Duffy (-)): 68% in African Americans and 88-100% in African people (including more than 90% of West African people). **Subjects under study:** In this retrospective cohort study, we analyzed the data from total patients with confirmed COVID-19 infection in the US through August 4, 2020.The US population 13.4% black non-Hispanic, 76.3% white non-Hispanic, Asian 5.9%, 18.5% Hispanic or Latino, and 4.3 all other races. The primary outcomes were hospitalization and in-hospital death. **Results:** A total of 4,923,692 patients in the US were tested Covid-19 positive and 155,459 mortality with a death rate of 3.15%. 11,383 deaths were excluded because they did not have racial details. Among the 144.076 patients who died of Covid-19, 73,095(50.6%) were white, 32,702(22.6%) were black, 27,387(19.2%) were Hispanic or Latino, 5,970(4.2%) were Asian and 7,045(4.8%) all other races. . The actual death rate per 100,000 is 35.9 in white, 80.4 in black, 45.8 in Hispanic or Latino, 33.1 in Asian, and 56.7 in all other races. **Conclusion**: Despite black Americans represent only 13.4% of the total population; they have been heavily hit by the COVID-19 pandemic. The reasons for the especially severe forms of the disease in Black Americans have not been fully uncovered. As we discussed before, the people with the Duffy (-) phenotype are a high-risk group for the Chemokine Storm. One hypothesis that arguably gives the blood group marker and chemokine decoy receptor Duffy protein which plays a central role in stimulating the Chemokine Storm as present in the severe forms of the COVID-19 disease.

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ARCHITECT / Alinity SARS-CoV-2 IgG and IgM Assays: Precise Fully Automated Methods for the Qualitative Detection of Human Antibodies to SARS-CoV-2

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Background: COVID-19 is a disease caused by a novel coronavirus now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus was first identified in December 2019 during an outbreak of respiratory illness in China. The incubation period of COVID-19 ranges between 1 and 14 days. The appearance and persistence of IgM and G antibodies allows identification of people who have been infected by the SARS-CoV-2 virus. The sensitivity of combining RNA with antibody results has been reported in the literature as >99%. The persistence of antibodies allows identification of people who have been recently infected and evaluation of disease courses including recovered from the illness. SARS-CoV-2 IgM, together with IgG serological assays, play an important role in research and surveillance.

Objective: To demonstrate the analytical performance of the SARS-CoV-2 Ig M and G Assays on the ARCHITECT and Alinity systems. The SARS-CoV-2 IgM / G Assays are two-step immunoassays for the qualitative detection of antibodies to SARS-CoV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Methods: Key performance criteria including precision, analytical specificity and clinical performance (Percent agreement) were assessed.

Results: The observed results for the Cutoff, precision (%CV), percent agreement are shown in the table below. Percent agreement studies demonstrated a positive percent agreement (PPA) at ≥ 7 days for IgM assay of 90.27% and >/= 14 days as 100% for the IgG assay. The Negative percent agreement (NPA) of 99.63% for IgG and 99.56% for IgM reflect the excellent overall specificity of the assays. Cross-reactivity was evaluated extensively for both assays on populations of other disease states and potential interferants.

	Cutoff S/C)	Precision (%CV)[a]	PPA	NPA
SARS-CoV-2 IgM Assay	< 1.00 Negative>/= 1.00 Positive	5% CV Negative Control2.9% CV Positive Control	90.27% (n=216)	99.56% (n=2965)
SARS-CoV-2 IgG Assay	< 1.40 Negative>/=1.4 Positive	5.9% CV Negative Control 1.2% CV Positive Control	100% (n= 88)	99.63% (n=1070)

Conclusion: The SARS-CoV-2 IgM and G Assays utilizing CMIA technology on the ARCHITECT and Alinity systems demonstrated acceptable performance for precision, specificity and percent agreement.

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Validation and Implementation of a Immunocromatographic Assay for the Identification of Donors Suitable for the Extraction of COVID 19 Convalescent Plasma

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Background: In March, the FDA released the use of convalescent plasma for USA in the context of clinical studies. In Chile at that time, no quantitative method was available to evaluate immunoglobulin concentration in COVID convalescent patients. Because there where no known treatment, nor vaccine, convalescent plasma transfusion could be an alternative for patients who have severe condition, or whith risk factors that promote serious disease. The correct choice of donors with adequate concentration of antibodies would allow an early apheresis and thus be able to make the plasma available for use. This is the experience of using an immunochromatographic test for the donor selection. Methods: All candidates for apheresis with more than 21 days of end of symptoms and positive PCR were tested with Juschek^{R} immunochromatographic test SARS-CoV-2 IgM/IgG test from serum with dilution 1/5 and 1/10 and subsequently compared with ELISA results. Sensitivity, specificity, negative (NPV) and positive predictive value (PPV) were evaluated to assess with what dilution it was possible to establish the cut-off point of 1/320 IgG antibody concentration allowing the rapid test to be used within the donor selection chain and thus to increase the efficiency of ELISA. Candidates who tested positive for the immunochromatographic test entered the apheresis process and the resulting plasma units were only released for transfusion when the quantitative ELISA result was given. Results: A total of 166 donors were tested, 100% with positive PCR for Sars-CoV 2. In patients with IgG antibody for Sars CoV-2 ratio greater than 1/320 by ELISA, dilution of 1/10 in rapid test gave sensitivity of 93% (104/112) and specificity of 70% (38/54). The positive predictive value was 87% (104/120) and the negative predictive value was 83% (38/46). In those patients with antibody ratio of 1/640 by ELISA the dilution of 1/10 in rapid test gave sensitivity of 97% (71/73) and specificity of 70% (38/54). The positive predictive value was 87% (71/87) and the negative predictive value was 95% (38/40). Conclusion: Even when the rapid tests are created to support the diagnosis of the disease, we have shown that a dilution of serum 1/10 with the Juscheck^{R} immunochromatographic test allowed to select with an appropriate sensitivity, specificity, PPV, and NPV, suitable donors for convalescent plasma apheresis at a time when the quantitative method was not so readily available. Having this tool allowed us to have plasma at the beginning of the pandemic in the country, being able to treat more than 300 patients with have severe condition, or whith risk factors that promote serious disease

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Performance of the Abbott SARS-CoV-2 Ig-G Assay

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Introduction: Antibodies to the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) increase 10-13 days after infection. We describe our evaluation of the Abbott SARS-CoV-2 IgG assay on the Architect immunoassay analyser.

Methods: We assessed assay precision, sensitivity (285 SARS-CoV-2 RT-PCR positive samples), specificity (718 pre-pandemic samples), and cross-reactivity to other positive antibody samples (influenza/dengue/hepatitis B and C/rheumatoid factor/anti-nuclear/double-stranded DNA/syphilis) on the Abbott SARS-CoV-2 IgG assay. The manufacturer cut-off index (COI) of 1.4 was adopted for positive results. The sample throughput of the Abbott assay was also assessed.

Results: The Abbott assay showed excellent precision, with a CV of 3.4% for the negative control (COI = 0.06) and 1.6% for the positive sample (COI = 8.6) respectively. The assay specificity was 100.0% (95% CI 99.49 to 100.00). There was minimal cross-reactivity with other antibodies, with 2 false positive cases (0.9%) for post-influenza sera (n=229). Of the 285 RT-PCR positive samples (from 160 individual patients), the PPA increased stepwise from 46.1% in week 1, to 83.0% by week 2 and 98.3% after 14 days post-positive RT-PCR (POS) (see Table 1). The Architect Abbott assay has a throughput of 100 samples in 70 minutes. Assuming a disease prevalence of 5%, in subjects ≥14 days POS, the positive predictive value of the Abbott assay is 100.0%, and the negative predictive value is 99.9% (95% CI 99.39 to 99.99). Residual serum was also available from inpatients not initially suspected of having COVID-19, 47.3% (35/74) of whom tested positive for SARS-CoV-2 IgG, indicating an early IgG response.

Conclusion: The Abbott SARS-CoV-2 IgG assay shows excellent performance that is within FDA and CDC guidelines when testing patients ≥14 days POS, with little cross reactivity from other antibodies. There is some evidence that SARS-CoV-2 IgG develops early in COVID-19.

TABLE 1: Abbott assay sensitivity (positive percentage agreement) by days post positive RT-PCR.

Days POS (N)	IgG positive/negative	PPA% (95% CI)
0-6 (178)	82/96	46.07 (38.59 to 53.68)
7-13 (47)	39/8	82.98 (69.19 to 92.35)
≥7 (107)	98/9	91.59 (84.63 to 96.08)
≥14 (60)	59/1	98.33 (91.06 to 99.96)

(Abbreviations: RT-PCR: Real-time polymerase chain reaction, POS: Post-positive RT-PCR, PPA: Positive percentage agreement, CI: Confidence interval)

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Routine Laboratory Biomarkers in COVID 19 Patients: Chasing the Storm

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Background: Coronavirus disease 2019 (COVID-19) is a global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As the number of individuals infected with COVID-19 continues to rise worldwide, as well as in India, it is clear that the routine laboratory will play an essential role in this crisis, contributing to patient monitoring, staging, prognosis and treatment guide. **Methods:** In this study, we evaluated the role of routine laboratory parameters: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum Albumin, Total Bilirubin (T Bil), Creatinine, C Reactive Protein (CRP), Procalcitonin (PCT), Lactate Dehydrogenase (LDH), D-Dimer, Interleukin 6 (IL6), Ferritin, Complete Blood Count (CBC) and Blood Gases in COVID 19 patients who are admitted with rRT-PCR positive test results in Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute, Mumbai, India. We explain one case of COVID 19 with cytokine storm here. **Results: :** We have observed statistically significant abnormal values of AST, ALT, T Bil, Creatinine, CRP, PCT, LDH, D-Dimer, IL6, Ferritin, Blood Gases, CBC and Albumin in COVID 19 patients. We obtained the laboratory data of cases admitted in Kokilaben Dhirubhai Ambani Hospital & Medical Research Institute. Severe COVID-19 associated pneumonia patients, with features of systemic hyper-inflammation, are designated under the umbrella term of macrophage activation syndrome (MAS) or cytokine storm. To explain the role of routine laboratory parameters in disease monitoring, we'll discuss routine lab parameters of one case here. An elderly female with a history of Hypertension presented to the hospital with a complain of breathlessness and fever since 2-3 days along with generalized weakness associated with giddiness. The patient had no travel or contact history. The patient was confirmed for COVID-19 by carrying out rRT-PCR testing for detection of SARS-CoV-2 using throat and nasal swab. On carrying out ECG, it showed ST waves and T waves changes in anterior and lateral leads. High Resolution CT scan (HRCT) showed bilateral subpleural ground glass opacities associated with small consolidations and crazy paving- mainly in the right lobe. The image features were typical for viral infection. The laboratory findings supported the diagnosis with elevated levels of ferritin (389.7ng/mL), IL-6 (9.83pg/ml), D Dimer (1555.94ng/mL), Creatinine (1.34mg/dl), hsTropinin I (130.6ng/L), CRP (3.33mg/dl). **Conclusion:** Therefore, routine laboratory biomarkers play an essential role in patient admission protocol, assessment of staging of disease according to severity, prognostication, patient monitoring and therapeutic monitoring.